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NIOSH GRANTS

COMPLETED
307

RESEARCH and DEMONSTRATION PROJECTS

ANNUAL REPORT
FISCAL YEAR 1985

ga/sw

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

NIOSH

RESEARCH AND DEMONSTRATION GRANTS

FISCAL YEAR 1985

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health
Atlanta, Georgia 30333

September 1986

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DHHS (NIOSH) Publication No. 86-123

FOREWORD

The National Institute for Occupational Safety and Health (NIOSH) plans, directs, and coordinates a national program of research, training, and related activities to prevent occupational diseases and injuries among workers in the United States. Under the provisions of the Occupational Safety and Health Act of 1970 and the Federal Mine Safety and Health Amendments Act of 1977, research investigations constitute a principal responsibility of NIOSH. In turn, the support of outstanding extramural research is a major component of this research program.

Since becoming Director of NIOSH, I have placed high priority on directing the Institute's activities toward the most significant problems in the field. To focus our intramural efforts, we developed a suggested list of the Ten Leading Work-Related Diseases and Injuries. These priorities are also reflected in our extramural research program, which is structured to make awards in each of the areas shown on page 4 of this document.

This report is published to provide a readily available source of information on the status and scope of the research grants program of NIOSH as a means of stimulating the development of proposals for research of high quality on significant occupational safety and health problems. In this we invite the interest of scientists in the biomedical sciences, engineering, and related disciplines. Descriptions of all active grants during fiscal year 1985 (October 1, 1984, to September 30, 1985) are included, along with publications resulting from the research.

J. Donald Millar, M.D., D.T.P.H. (Lond.)
Assistant Surgeon General
Director, National Institute for
Occupational Safety and Health
Centers for Disease Control

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ANNOUNCEMENT

RESEARCH AND DEMONSTRATIONS RELATING TO OCCUPATIONAL SAFETY AND HEALTH

DEPARTMENT OF HEALTH AND HUMAN SERVICES

CENTERS FOR DISEASE CONTROL

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

Application Receipt Dates: New applications (February 1, June 1, October 1); Exceptions: Career Development, Small Grants, and Competing renewal applications (March 1, July 1, November 1).

The Centers for Disease Control (CDC), National Institute for Occupational Safety and Health (NIOSH) announces that competitive grant applications are being accepted for research and demonstrations relating to occupational safety and health. These include innovative methods, techniques, and approaches for dealing with occupational safety and health problems in the general industry and in mining industry.

Support in the form of project grants will be awarded for annual budget periods, within a project period not to exceed five years.

I. AUTHORITY

These grants will be awarded and administered by NIOSH under the research and demonstration grant authority of Section 20(a)(1) of the Occupational Safety and Health Act of 1970 (29 U.S.C. 669(a)(1) and Section 501(c) of the Federal Mine Safety and Health Amendments Act of 1977 (30 U.S.C. 951). Program regulations applicable to these grants are contained in Part 87 of Title 42, Code of Federal Regulations, "National Institute for Occupational Safety and Health Research and Demonstration Grants." Except as otherwise indicated, the basic grant administration policies of the Department of Health and Human Services and the Public Health Service are applicable to this program.

II. ELIGIBLE APPLICANTS

Eligible applicants include non-profit and for-profit organizations. Thus universities, colleges, research institutions and other public and private organizations including State and local governments and small, minority and/or woman-owned businesses are eligible for these research and demonstration grants. For-profit organizations will be required to submit a certification as to their status as part of their application.

III. PROGRAM REQUIREMENTS

A. Research Project Grants

A research project grant application should be intended and designed to establish, discover, develop, elucidate, or confirm information relating to occupational safety and health, including innovative methods, techniques, and approaches for dealing with occupational safety and health problems. These studies may generate information that is readily available to solve problems or contribute to a better understanding of underlying causes and mechanisms.

B. Demonstration Grants

A demonstration grant application should address, either on a pilot or full-scale basis, the technical or economic feasibility or application of: (1) a new or improved occupational safety or health procedure, method, technique, or system, or (2) an innovative method, technique, or approach for preventing occupational safety or health problems.

C. Special Emphasis Research Career Award (SERCA) Grants

The SERCA is designed to enhance the research capability of individuals in the formative stages of their careers who have demonstrated outstanding potential for contributing as independent investigators to health-related research. Candidates must have had two or more years of relevant postdoctoral experience prior to the submission date. The application must document accomplishments in this period that demonstrate research potential; it must also present a plan for additional experience in a productive scientific environment at domestic institutions that will foster development of a career of independent research in the area of occupational safety and health. The SERCA is not intended for untried investigators, or for productive, independent investigators with significant numbers of publications of high quality, or for persons of senior academic rank (above associate professor or tenured). Moreover, the award is not intended to substitute one source of salary support for another for an individual who is already conducting full-time research, nor is it intended to be a mechanism for providing institutional support. The application must demonstrate that the award will make a difference in and enhance the candidate's development as an independent investigator.

Candidates must indicate a commitment of at least 60 percent time (not necessarily 60% salary) devoted to research under the SERCA grant, although full-time is desirable. Other work in the area of occupational safety and health will enhance the candidate's qualifications but is not a substitute for this requirement. While working closely with one or more advisers, the awardee is expected to develop capabilities in fundamental, applied, and/or clinical research in one of the areas in section IV. At the end of the award period, evidence of independent investigative capability should be present such that the individual is better able to compete in traditional NIOSH research grant activities.

The total grant award may comprise direct costs of up to $30,000 per year and up to eight percent additional indirect costs. Direct costs may include salary plus fringe benefits, technical assistance, equipment, supplies, consultant costs, domestic travel, publication, and other costs. If the awardee already holds a small grant on the same research topic, the amount of the SERCA may be reduced up to the amount of the small grant. Awards may be up to three years and will not be renewable.

D. Small Grants

A small grant application is intended to provide financial support to carry out exploratory or pilot studies, to develop or test new techniques or methods, or to analyze data previously collected. This small grant program is intended for predoctoral graduate students, post-doctoral researchers (within three years following completion of doctoral degree or completion of residency or public health training) and junior faculty members (no higher than assistant professor). If university policy requires that a more senior person be listed as principal investigator, the application should specify that the funds are for the use of a particular student or junior-level person and should include appropriate justification for this arrangement. Though biographical sketches are required only for the person actually doing the work, the application should indicate who would be supervising the research. Small grant applications should be identified as such on the application form.

The total small grant award may comprise direct costs of up to $15,000 per year and additional indirect costs, as appropriate. The grants may be awarded for up to two years and are thereafter continuable by competitive renewal as a regular research grant. Salary of the principal investigator as well as that of the junior investigator, if university policy requires a senior person to be listed as the principal investigator, will not be allowed on a small grant, though salaries can be requested for necessary support staff such as laboratory technicians, interviewers, etc.

E. Program Project Grants

NIOSH will also accept applications for program project grants, but only after discussion with the individuals listed in this announcement.

IV. PROGRAMMATIC INTEREST

Work-related concerns of programmatic interest to NIOSH are listed below, which are applicable to all of the above types of grants. The conditions or examples listed under each category are selected examples, not comprehensive definitions of the category. Investigators may also apply in other areas related to occupational safety and health. Applications responding to this announcement will be reviewed by staff for their responsiveness and relevance to occupational safety and health. Assignment to NIOSH for funding consideration will be according to established referral guidelines. Potential applicants with questions concerning the acceptability of their proposed work should contact the individuals listed in this announcement under "FOR FURTHER INFORMATION CONTACT."

1. Occupational lung disease: asbestosis, byssinosis, silicosis, coal workers' pneumoconiosis, lung cancer, occupational asthma

2. Musculoskeletal injuries: disorders of the back, trunk, upper extremity, neck, lower extremity: traumatically induced Raynaud's phenomenon

3. Occupational cancers (other than lung): leukemia; mesothelioma; cancers of the bladder, nose and liver

4. Severe occupational traumatic injuries: amputations, fractures, eye loss, and lacerations

5. Cardiovascular diseases: hypertension, coronary artery disease, acute myocardial infarction

6. Disorders of reproduction: infertility, spontaneous abortion, teratogenesis

7. Neurotoxic disorders: peripheral neuropathy, toxic encephalitis, psychoses, extreme personality changes (exposure-related)

8. Noise-induced loss of hearing

9. Dermatologic conditions: dermatoses, burns (scalding), chemical burns, contusions (abrasions)

10. Psychologic disorders: neuroses, personality disorders, alcoholism, drug dependency

11. Control technology research: application of scientific principles to control strategies; preconstruction review; technology forcing/new source performance concepts; technology transfer; substitution; unit operations approaches

12. Respirator research: new and innovative respiratory protective devices; techniques to predict performance; effectiveness of respirator programs; physiologic and ergonomic factors; medical surveillance strategies, psychological and motivational aspects; Effectiveness of sorbents and filters, including chemical and physical properties

V. CRITERIA FOR REVIEW

Applications will be evaluated by a dual review process. The primary (peer) review is based on scientific merit and significance of the project, competence of the proposed staff in relation to the type of research involved, feasibility of the project, likelihood of its producing meaningful results, appropriateness of the proposed project period, adequacy of the applicant's resources available for the project, and appropriateness of the budget request.

Demonstration grant applications will be reviewed additionally on the basis of the following criteria:

- Degree to which project objectives are clearly established, obtainable, and for which progress toward attainment can and will be measured.
- Availability, adequacy, and competence of personnel, facilities, and other resources needed to carry out the project.
- Degree to which the project can be expected to yield or demonstrate results that will be useful and desirable on a national or regional basis.
- Extent of cooperation expected from industry, unions, or other participants in the project, where applicable.

SERCA grant applications will be reviewed additionally on the basis of the following criteria:

- The review process will consider the applicant's scientific achievements, evidence of demonstrated commitment to a research career in occupational safety and health, and supportive nature of the research environment (including letter(s) of reference from advisor(s) which should accompany the application).

Small grant applications will be reviewed additionally on the basis of the following criteria:

- The review process will take into consideration the fact that the applicants do not have extensive experience with the grant process.

A secondary review will also be conducted. Factors considered in the secondary review will include:

- The results of the initial review.
- The significance of the proposed study to the research programs of NIOSH.
- National needs and program balance.
- Policy and budgetary considerations.

VI. APPLICATION AND AWARD

Applications should be submitted on Form PHS-398 (revised May 1982) or PHS-5161-1 for State and local government applications. Forms should be available from the institutional business offices or from:

Office of Grants Inquiries
Division of Research Grants
National Institutes of Health
Westwood Building - Room 449
5333 Westbard Avenue
Bethesda, Maryland 20205

The original and six copies of the application must be submitted to the address below on or before the specified receipt dates in accordance with the instructions in the PHS-398 packet:

Division of Research Grants
National Institutes of Health
Westwood Building - Room 240
Bethesda, Maryland 20205

In developing the application please note that the conventional presentation for grant applications should be used and the points identified under "CRITERIA FOR REVIEW" must be fulfilled.

An applicant organization has the option of having specific salary and fringe benefit amounts for individuals omitted from the copies of the application that are made available to outside reviewing groups. If the applicants organization elects to exercise this option, use asterisks on the original and six copies of the application to indicate those individuals for whom salaries and fringe benefits are being requested; the subtotals must still be shown. In addition, submit an additional copy of page four of Form PHS-398, completed in full with the asterisks replaced by the amount of the salary and fringe benefits requested for each individual listed. This budget page will be reserved for internal PHS staff use only.

The instructions in the Form PHS-398 packet should be followed concerning deadlines for either delivering or mailing the applications. The application should be sent or delivered using the mailing label in the Form PHS-398 packet.

The proposed timetable for receiving applications and awarding grants is as follows:

New applications:

Application Deadline	Primary Review Group Meeting	Secondary Review Meeting	Expected Start Date
February 1	June	September	December 1
June 1	Oct./Nov.	January	April 1
October 1	Feb./Mar.	May	July 1

Exceptions: Career Development, Small Grants, and Competing renewal applications:

Application Deadline	Primary Review Group Meeting	Secondary Review Meeting	Expected Start Date
March 1	June	September	December 1
July 1	Oct./Nov.	January	April 1
November 1	Feb./Mar.	May	July 1

Awards will be made based on priority score ranking and emphasis area, as well as availability of funds.

VII. REPORTING REQUIREMENTS

Performance reports on awarded grant projects are required annually as a part of the continuation application and a final report is due within ninety days of the end of the project period. The final perforamnce report should include, at a minimum, a statement of original objectives, a summary of research methodology, a summary of positive and negative findings, and a list of publications resulting from the grant. Research papers, project reports, or theses are acceptable itmes in the final report. The report should stand alone rather than citing the original application. Three copies of reprints of publications prepared under the grant should accompany the report.

VIII. FOR FURTHER INFORMATION CONTACT:

For Technical Information Contact:
Roy M. Fleming, Sc.D.
Associate Director for Grants
National Institute for Occupational Safety and Health
Centers for Disease Control
1600 Clifton Road, N.E., Bldg. 1, Room 3053
Atlanta, Georgia 30333
Telephone: (404) 329-3343

For Business Information Contact:
Leo A. Sanders
Grants Management Officer
Centers for Disease Control
255 E. Paces Ferry Rd., NE, Room 321
Atlanta, Georgia 30305
Telephone: (404) 262-6575

(This program is described in the Catalog of Federal Domestic Assistance Program No. 13.262, Occupational Safety and Health Research Grants. It is not subject to Health Systems Agency nor E.O. 12372 review.)

GLOSSARY

Grant Number - The identification number assigned to a grant application

EXAMPLE: 1 R01 OH02183-01A1

```
1   R01   OH   02183   -01   A1
|    |    |      |      |    |
|    |    |      |      |    |_ Signifies a first resubmission with
|    |    |      |      |       modifications
|    |    |      |      |_ Year of funding
|    |    |      |
|    |    |      |_ Sequence number for NIOSH grants
|    |    |
|    |    |_ Institute with primary assignment
|    |
|    |_ Grant Activity
|
|_ Grant Type
```

Typical Codes
- Grant Type
  - 1 - New Competing application
  - 2 - Competing renewal application
  - 5 - Noncompeting continuation application
- Grant Activity
  - R01 - Research Project Grant
  - K01 - Special Emphasis Research Career Award (SERCA) Grant
  - R03 - Small Grant
  - R13 - Conference Grant
  - R18 - Demonstration Grant
  - R43 - Small Business Innovation Research (SBIR) Grant
- Institute
  - OH - NIOSH

Program Areas - Research categories of particular interest to NIOSH

Occupational Lung Diseases	(Lung)
Musculoskeletal Injuries	(Muscu)
Occupational Cancers	(Cancer)
Traumatic Injuries	(Trauma)
Cardiovascular Diseases	(Cardio)
Reproductive Disorders	(Repro)
Neurotoxic Disorders	(Neuro)
Noise-Induced Hearing Loss	(Noise)
Dermatologic Conditions	(Derma)
Psychologic Disorders	(Psycho)
Control Technology Research	(Contr)
Respirator Research	(Respir)
Other Occupational Concerns	(Other)

AEROSOL DEPOSITION IN THE HUMAN RESPIRATORY SYSTEM

C.P. Yu, Ph.D.
Department of Mechanical and Aerospace Engineering
State University of New York at Buffalo
Clifford C. Furnas Hall
Amherst, New York 14260

Occupational Lung Diseases
5 R01 OH00923-05
09/30/83 - 08/31/86
$58,351 ($215,528 Cum)

Objectives

This research is directed toward determining the amount and site(s) of deposition of inhaled particles and their subsequent fast clearance in the human respiratory system with the use of theoretical and computational models. Special efforts will be made to examine (i) the effects of various particle factors such as size, shape, charge, mass density and hygroscopicity on deposition and (ii) the extent and causes of intersubject variabilities.

Methodology

Analytical and statistical methods will be employed to achieve the goals with extensive reference to available experimental data. Computer programs are to be developed for predicting deposition under various exposure conditions.

Progress & Accomplishments

At this time, we have completed the following major studies:

1. Modeling of head deposition for spherical and fibrous particles.
2. Development of lung deposition models involving:
   a. derivation of deposition efficiency formulas in an airway due to various mechanisms;
   b. Study of particle charge effects;
   c. deposition of ultrafine particles;
   d. deposition of fibrous particles;
   e. deposition of hygroscopic particles;
   f. deposition in young age groups.
3. Development of probabilistic deposition models for:
   a. deposition of polydisperse aerosols;
   b. lung deposition in different anatomical models;
   c. statistical deposition models with random airway structures.
4. Determination of mucociliary transport rates in bronchial airways and the effect of sulfuric acid on such rates.

Significance

Airborne toxic particulates, inhaled into the respiratory system, will deposit there and produce detrimental biological effects. Since the complete removal of these particulates from the industrial environment is practically impossible and economically unfeasible, it is necessary to establish certain exposure standards for occupational protection. It is hoped that within these standards, any biological damage would be unlikely even after long periods of exposure. The results of this research will lead to a better fundamental understanding of the dose-response relationship for occupationally-induced lung diseases and thus may serve as a basis for developing an effective prevention strategy.

Publications

Diu CK, Yu CP: Deposition from Charged Aerosol Flows through a Two-Dimensional Bend. J Aerosol Sci 11:383, 1980.

Diu CK, Yu CP: Deposition from Charged Aerosol Flows through a Pipe Bend. J Aerosol Sci 11:397, 1980.

Chan TL, Yu CP: Charge Effects on Particle Deposition in the Human Tracheobronchial Tree. Fifth International Symposium on Inhaled Particles, Wales, England, September 1980, Ann Occup Hyg 26:65, 1982.

Yu CP, Diu CK, Soong TT: Statistical Analysis of Aerosol Deposition in Nose and Mouth. Am Ind Hyg Assoc J 42:726, 1981.

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HEALTH RISKS IN THE VERMONT GRANITE INDUSTRY

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Occupational Lung Diseases
5 R01 OH01035-03
04/01/83 - 03/31/87
$262,816 ($755,042 Cum)

Objectives

The overall aim of this study is to carry out a comprehensive assessment of health risks in the Vermont Granite industry, which involves exposure to low levels of quartz. The major specific objectives are three:

1. To perform a longitudinal study of pulmonary function losses in the current workforce. Previous estimates of losses suggested that the loss of forced vital capacity and FEV_1 in this group of workers was excessive and was related to occupational dust exposure. Certain doubts were raised about these previous studies and the current effort is an attempt to confirm, deny, or qualify the suggestion that excessive loss occurs independent of smoking or aging.

2. The second objective is to carry out an industry wide radiographic survey to ascertain the prevalence of abnormalities consistent with pneumoconiosis in workers who have been exposed to low levels of granite dust since 1940. Radiographic surveys of the industry have been done by the Vermont Industrial Hygiene Division since 1940, suggesting as of 1965 that silicosis was being controlled, but studies published in the 1970's stated that a 30% prevalence of abnormalities existed. The current effort is to assess whether radiographic silicosis or silicotuberculosis exist and to what degree. Many of the workers studied will have a prolonged exposure to granite dust (more than 30 years). Those workers showing abnormalities will be compared with their normal colleagues in terms of smoking history and dust exposure to ascertain the significance of the radiographic changes.

3. The third aim is to make systemic measurements of dust levels in different size stone sheds and in different occupational categories within the industry.

A subsidiary objective includes a study of retired granite workers both with and without radiographic silicosis who have been followed longitudinally both by x-ray and pulmonary function testing. The purpose of this aspect of the project is to ascertain the rate of which pulmonary function changes occur in men who are no longer occupationally exposed to dust and to compare rates of pulmonary function decrement in those with and without silicosis.

Methodology

For spirometric testing, we have used a Collins 13 liter water-sealed Stead-Wells type apparatus since 1979. Testing is carried out at the workplace. Guidelines set down by the American Thoracic Society are being followed in terms of evaluating the results and the acceptability of tracings.

Collection of 8-hour dust samples has utilized personal size selective samplers running at 2 liters per minute for gravimetric estimation. The samples are being analyzed for percentage quartz using x-ray defraction techniques.

Chest x-rays are being read by three certified B readers using the ILO/UC classification system. The definition of abnormality we propose to use is based on a profusion reading of 1/0 or greater of densities considered to be consistent with pneumoconiosis by either two or three of the readers. These are of course analyzed as to type of abnormality and location as prescribed by the format.

Progress & Accomplishments

Spirometric surveys have now been carried out four times, including two industry wide surveys which antedated the beginning of the project, which were done in 1979 and 1981. Subsequent surveys have been done in the Spring of 1983 and 1985. Participation of the work force appears to be high. These results have not been analyzed as yet longitudinally, though the data has been entered in the computers and is nearly ready for analysis. We anticipate carrying out a final survey in the Spring of 1987 which will provide an 8-year period of observation of this group of workers.

Approximately 450 dust samples have been collected in various sheds and assorted occupations. The average dust level is 581 mcg/m^3; the range of dust exposure varies from 10 to over 2000 mg/m^3. These values are comparable to those observed by previous investigators and by the Vermont Occupational Safety and Health Administration. Depending on occupational category, the average values for total dust varied from a low of 389 mcg/m for boxers to a high of 912 mcg/m^3 for maintenance workers (N-6). The roentgenographic survey carried out in 1983 numbered approximately 970 workers and this material has been interpreted and analyzed. Only 26 films were considered to be abnormal as defined by 2 or 3 readers seeing opacities consistent with pneumoconiosis in a profusion of 1/0 or greater. However, 20 of these were abnormalities of the irregular or reticular type and appeared to be predominantly lower lobe.

In addition, we have either x-rayed recently or found films on an additional 1000-2000 former or retired workers who were no longer employed during the survey of 1983. These films are being interpreted and coded and will be analyzed in a comparable manner. The total x-ray analysis should include close to 90% of all Vermont stone-shed workers employed since 1940 and exposed to low dust levels.

Significance

The usefulness of this information pertains mainly to the issue of a safe occupational level of quartz exposure. Currently, the Department of Labor accepts a level of 100 mcg/M^3 of quartz, whereas NIOSH has recommended a level of 50 mcg/M^3. The levels of dust that we have measured are certainly above the NIOSH recommended level and may be considerably higher than the current levels, based on our estimate of 18% quartz using x-ray diffraction classification. Based on the data so far, it appears that the level of radiographic silicosis is extremely low and there appeared to be no large opacities suggesting complicated disease nor any tuberculosis. The issue whether contemporary dust levels are leading to an excessive loss of pulmonary function should be possible to extract from the data since we will have internal controls. Thus, we will be able to compare granite workers exposed to a given level of dust and of comparable lengths of exposures among smokers and non-smokers.

Publications - None to Date

ACTIVITY OF AMOSITE ASBESTOS AND GRUNERITE FIBER

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Occupational Lung Diseases
5 R01 OH01151-02
09/01/82 - 08/31/85
$64,355 ($125,161 Cum)

Objectives

The goal was to obtain a thorough understanding of amosite (asbestiform grunerite) in terms of its physical and chemical properties and morphology and how these data compare to those collected on non-asbestiform grunerite. Lung tissues from workers who have died from asbestos-related diseases contain particles of widely varying morphologies, so the present study has sought to examine the connection between particle morphology and method of comminution which could be applied to the larger question of the biological activity of amosite and non-asbestiform grunerite and the etiology of asbestos disease. Due to the significance of crocidolite in the epidemiology of mesothelioma. comparative studies of the properties of crocidolite (and non-asbestiform riebeckite) to those of amosite/grunerite were added to the goals of the project.

Methodology

Samples were obtained in an uncomminuted state (i.e., as rocks) and examined before and after various degrees and styles of comminution. UICC samples were also available and studied. The texture and mineral assemblage of the host rock were observed in petrologic thin sections of specimens using polarized-light microscopy. Chemical compositions of amphibole and associated minerals were determined by electron-probe microanalysis (5-10 micron spatial resolution). Comminution techniques included grinding (or milling) and sonication (disaggregating with an ultrasonic cell-disrupter), since each method produces particles with significantly different morphology. Both transmission electron microscopy (TEM) and selected area electron diffraction (SAED) was carried out on both sonicated and milled particles, specifically to examine particle shape and size, preferred orientation on the sample grid, and defect structures. Particle size distributions were measured on enlarged electron micrographs using a digitizing table and computer program. Semiquantitative analytical electron microscopy was used to establish the chemical compositional identity of particles and to compare with microprobe and published data. Particle morphology was also studied using scanning electron microscopy (SEM), specifically for examining particle cross sections. Single-crystal X-ray diffraction was used to obtain standard diffraction patterns. X-ray powder diffractometery was used (and is still underway) to determine whether characteristics such as lattice parameters or spectral sharpness could be used routinely to differentiate between powdered amosite and grunerite samples.

Progress & Accomplishments

A suite of amosite and grunerite samples was assembled including 5 amosite specimens from South Africa. Six specimens of crocidolite were examined, including samples from both the Cape Province and Transvaal of

South Africa; the later locality produces mixed amosite and crocidolite. Chemical analysis shows whereas amosite is restricted in composition, typical grunerite shows a considerable range. A sample from Lead, South Dakota is of interest because its composition is similar to that of amosite and because there is workplace exposure to dusts of this grunerite at the Homestake Mine. Analyses of crocidolite show greater variation than amosite; a comparable non-asbestiform riebeckite source being St. Peter's Dome, Colorado.

The physical features of amosite dust particles depend on the processing technique used in comminution. Uncomminuted fibers consist of bundles of aligned fibrils, their c-axes parallel. Simple disaggregation produces fibers consisting of one or more individual amphibole prisms bounded by many faces, often with reentrant angles. Sonicated amosite fibers separate into their constituent fibrils which are generally rounded in cross-section. Fine twin lamellae, on the (100) crystallographic plane, are observable by diffraction contrast and vary in thickness from 40Å to 200Å. Grinding (or milling) amosite produces very thin, lath-shaped dust particles. Twinning in amosite fibrils and the prominent (100) surfaces suggests parting along twin boundaries. Grunerite dust particles, in contrast with amosite, are bound primarily by the standard (110) amphibole cleavage planes and tend to be substantially thicker and more irregular in shape.

Size distributions were evaluated by measuring length and width of fine particles (on TEM grids) that were comminuted for increasing lengths of time in an agate mortar. The salient features are the (1) these observations approximate a log-normal distribution, (2) there appears to be a unit fibril width that is unaffected by comminution and that has an average value of approximately 0.3 microns for amosite and 0.06 microns for crocidolite, (3) large fibers are the most affected by crushing in the mortar, their proportion and length decreases with increasing duration of comminution, and (4) short fibers (less than 5 microns) generally increase in number without altering the general size distribution of the particle population. Data for sonicated fibers are incomplete; however, diameters of such fibers are forthcoming. The most obvious difference between the lead grunerite data and the amosite data is that the mean aspect ratio is greatly reduced for Lead grunerite, from 11.6 for 1 minute or 5.8 for 20 minutes for amosite to 3.9 for 10 minutes and 2.3 for 30 minutes for crushed lead grunerite of respirable diameter. Comparable differences are observed in the data for riebeckite and crocidolite, which are forthcoming.

Significance

Several important features of these data relate to the questions about the activity of asbestos.

1. The crystallographic nature of the exposed surface: The surface of comminuted amosite is different from that of uncomminuted amosite or crushed grunerite.

2. Long particles: These are greatly reduced in number in highly comminuted amosite and are almost entirely absent from respirable Lead grunerite.

3. Short particles: Narrow unit fibrils and short ones (less than 5 microns) are greatly increased by comminution; if exposure to these is significant to disease, then highly processed amosite will produce a greater risk.

4. Smallest dimension: Parting of amosite on (100) produces very thin but normally wide fiber, so the smallest fiber dimension is enhanced for comminuted amosite.

Measurement of fiber dimensions has always been an important problem. Light microscopy has been preferred, however, fibers with submicron widths are virtually invisible in light optics. Small fiber distributions have been extrapolated from large particles sizes measured with light microscopy assuming a normal size distribution which is not the case. Even the relatively large-fiber size distributions are not adequately determined with light microscopy. With respect to this problem of asbestos particle definition, it is obvious that a 3 to 1 aspect ratio will not distinguish asbestiform from non-asbestiform grunerite except in the most highly comminuted, submicroscopic sizes. A more reasonable test for asbestiform grunerite is an aspect ratio of 10 to 1, where there is good statistics to show that asbestos has truly been found. In particular, this will most likely hold true for fibers visible with the light microscope.

Publications

Harlow GE, Kimball MR, Dowty E, Langer AM: Observations on amosite/grunerite dusts. In Applied Mineralogy Proceedings of the International Congress on Applied Mineralogy in the Mineral Industry, February 22-25, 1984, p 1147-1157.

FIBER DEPOSITION IN HUMAN UPPER AIRWAY MODEL

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Occupational Lung Diseases
5 R01 OH01605-03
09/30/83 - 08/31/86
$69,061 ($190,558 Cum)

Objectives

The hypothesis to be tested in this study is that the human upper respiratory airways play a significant role in the mode of exposure to airborne fibrous materials. Fibers removed in the nasal or oral airways are not accessible to the deep lung and this can play no role in chronic respiratory disease. Thus sampling of airborne fibers for assessment of risk of respiratory diseases ought to include those lengths and diameters accessible to the non-ciliated airways. The overall aim of the study is to assess the effects of fiber physical characteristics, mode of breathing, and breathing parameters on upper respiratory tract fiber deposition and compare these effects with those for non-fibrous particles.

Methodology

Since no experimental measurements of fiber removal in the human upper airway have been reported, the approach of this study is experimental in nature. While it would be ideal to carry out the studies in human subjects, this is not possible for fibers because of the unknown toxicity of low exposures of glass fiber. Thus, the approach adopted for the experimental measurement of overall and regional removal of fibers in the human upper respiratory airways is to employ an accurate physical model through which fiber aerosols are passed under flow conditions simulation various condition of breathing. The fibers used in the study are grouped according to narrow ranges of diameter and lengths adjusted by pretreatment. Fibers are labeled with a short-lived radioisotope so that small fiber masses may be measured both within and in the effluent stream of the model. Experiments with monodisperse spherical aerosols have been carried out in similar models for local nasal deposition for comparative purposes.

Progress & Accomplishments

The initial experimental studies were with monodisperse spherical aerosols of diameter 0.4, 1.2, 4.0 um diameter at flow rates corresponding to resting respiration in a nasal passage mode. Local deposition of aerosol was primarily in the anterior region for the 1.2 and 4.0 um aerosol because inertial impaction was the primary deposition mechanism. For the 0.4 unaerosol, the deposition pattern was much more diffuse, consistent with the mechanism of diffusion. The overall fractional deposition for 1.2 um aerosol was 1.5%, while for 4.0 um it was 30% and for 0.4 um it was 1.0%. Overall deposition experiment with intermediate diameter aerosols demonstrated a minimum deposition of 0.6% at 0.9 um.

Comparison experiment with three graded fiber aerosols were carried out in the nasal model. The fiber diameter were 0.3, 0.6, and 3.0 um and lengths were from 10-30 um. The 0.3 um fiber diameter aerosols had 8% deposition, while the 0.6 um fiber diameter had 3.5% deposition and the .30 um diameter

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fiber had 6%. Similar to the spherical aerosols, there appears to be a minimum deposition near 1.0 um, but the fraction deposition comparing spherical particle diameters to fiber diameter, is greater for the submicrometer aerosols and less for the aerosols greater than 1 um. Regional deposition of these fiber aerosol is being determined by profile scanning in two dimensions.

Oral passage deposition experiments in the model are beginning. The results of these studies will be compared to the human experimental results carried out previously in our laboratories. It is expected that oral deposition of fibers will be less than nasal deposition, but the degree of difference cannot be predicted. When the data is obtained for all the fibers and conditions, we plan to develop empirical equations which can be used to predict penetration of fiber aerosols as a function of fiber physical properties, mode of breathing (oral, nasal, or oronasal), and level of exercise (breathing, volume, and rate).

Significance

Major efforts are being made to identify, quantify, and control exposure to airborne fibers. A primary component of this exposure is inhalation, and the respiratory diseases associated with inhalation require deep lung deposition and lengthy retention. For fiber particles to cause such respiratory diseases, they must penetrate the upper airways. In order to correctly assess the risk of exposure, the fibers most likely to reach the deep lung must be known. With the information from this study, it will be possible to predict which airborne fibers will most effectively reach the deep lung and which fibers will deposit in the upper airways where they can be cleared to the G-I tract and may be involved in other disease process. Any fibrous aerosols, such as glass fiber, amphibole asbestos, or carbon fiber should be subject to the same empirical equations for predicting deep lung delivery as a function of fiber diameter and length.

Publications

Itoh H, Smaldone GC, Swift DL, Wagner HN: Mechanisms of Aerosol Deposition in a Nasal Model. J Aerosol Sci 16:529-534, 1985.

RESPIRATORY HAZARDS OF POULTRY WORKERS

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Occupational Lung Diseases
5 R01 OH02029-02
09/01/84 - 08/31/88
$208,958 ($299,589 Cum)

Objectives

We propose the first indepth medical and environmental study to investigate possible occupational hazards of poultry workers. Our research has five major aims:

To characterize the operations within the various components of the poultry industry on a national basis, relative to occupational health risks.

To identify the significant subgroups of poultry workers at potential risk.

To evaluate the air in the work environment of selected establishments for potentially harmful vapors and dusts.

To make any indicated recommendations to the poultry industry for improvement of the workplace and worker protection.

Methodology

The proposed investigation is a three-phase four-year study. Phase I is a 12-month effort to develop an industry-wide worker exposure profile by performing a series of walkthrough evaluations of typical turkey-growing, chicken-growing, hatcher, egg production, and poultry processing operations. We intend to identify and quantify the subgroups of poultry workers with the greatest potential for occupational health risks for comprehensive medical and environmental studies in phase II.

In phase II (years 2 and 3) we will recruit 400 workers for medical studies, (100 workers from each of the 4 subgroups of poultry workers at greatest risk). Medical assessment of these subjects will include ATS questionnaire (modified for occupational history), spirometry before and after 6 hours of work, serum precipitins, and skin testing. These data will be compared to two reference populations: 1) regionally proportioned unexposed blue collar workers, and 2) a blue collar reference population previously studied by NIOSH. Environmental assessment of the estimated 116 work-sites and 20 control work-sites will include measurements for ammonia, carbon dioxide, carbon monoxide, total and respirable dust, and endotoxin.

Phase III, data analysis, will occur during year 04 to the study. We will examine the relationships of environmental exposures to respiratory symptoms and pulmonary function. We will also examine relationships of environmental control systems and levels of environmental contaminants. We will determine what recommendations are needed if any, to improve the work environment, to protect workers from potential health hazards, and for possible future research.

Progress & Accomplishments

Phase I of the project includes both data collection and data generation activities. A comprehensive literature review has been underway and is continuing. This review involves production figures, processes, structures, work practices, a general assessment of potential occupational exposures, and geographical influences on those characteristics. The goal of this phase is to determine the size of the population at risk and to get an idea of air contaminants that may be encountered in phase II.

Walkthrough assessments of poultry operations in Iowa, Minnesota, Arkansas, and California have been completed. Data has been collected concerning concentration of airborne dust and ammonia. Types of building design with the inherent variables of ventilation and bird handling have been ascertained. With these data in hand a risk index is being developed to qualify those groups with greatest exposure and potential occupational hazards.

Also in this phase recruitment of study subjects for phase II is being undertaken. This is a key element of the success of this study and progress has been made procuring mailing lists, poultry extension specialists cooperation, and management of some poultry processing companies have been receptive to the idea of the study. Mailing lists have been utilized to sample survey populations of poultry workers.

Significance

Agricultural workers are a large and understudied population. Over the past few years we have recognized an emerging occupational respiratory disease hazard to farmers and farm workers within swine confinement buildings. Since most of the poultry in the U.S. are now raised in confinement buildings, we anticipate similar occupational problems may be present in poultry workers. Our pilot studies suggested they experience exposures and respiratory symptoms similar to swine confinement workers.

Publications - Abstract(s) available

PULMONARY TOXICITY OF THE SEMICONDUCTOR, GALLIUM ARSENIDE

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College of Pharmacy	11/01/84 - 10/31/87
University of Arizona	$112,239 ($112,239 Cum)
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Objectives

The objective is to investigate the lung toxicity and potential systemic toxicity of particulate gallium arsenide (GaAs), a semiconductor in industrial use. Previous work has shown a dose-dependent increase in rat lung weight after intratracheal GaAs that was not caused by aqueous edema. These lungs showed histological changes after 14 and 28 days but fibrosis was not evident from the histology nor were 4-hydroxyproline levels elevated.

The aims of this study are to define the lung changes induced by GaAs and to choose an animal model other than the rat for these studies. Another animal model will be chosen based on its sensitivity to indicators of systemic arsenic toxicity. The lung toxicity will be examined at times up to 6 months after exposure to inratracheal GaAs to determine if significant changes have occured in lung lipids, collagen, protein or DNA. These changes will be correlated with tissue and blood levels of gallium and arsenic.

Methodology

The response of the rat to arsenic will be compared to the mouse, hamster and guinea pig. The systemic toxicity parameters examined include: hemoglobin, blood and fecal arsenic, plasma glucose, creatinine and urea nitrogen, urinary porphyrin and kidney pyruvate dehydrogenase. The sensitive parameters will be chosen and dose-response relationships determined.

The lung toxicity of GaAs after intratracheal administration will focus on changes in collagen and histology. Collagen synthesis and degredation and proline pool size changes induced by 2 dose levels of GaAs will be compared with those caused by silica. Lipid changes that may be studied are phospholipids, cholesterol and fatty acids. Other parameters will be examined to determine the overall lung response: lung wet weight, lung dry weight, protein and DNA. Arsenic and gallium levels in lung, blood and excreta will be measured. Arsenic will be determined by atomic absorption spectrophotometry after arsine generation and gallium will be determined by fluorescence spectrophotometry after chelation or flame emission methods.

Progress & Accomplishments

The animal species were compared and the mouse showed the best response to arsenic. Even though arsenic inhibits many enzymes in vitro, only pyruvate dehydrogenase and urinary porphyrins were sensitive to arsenic at the 0.1 and 1.0 mg/kg doses used. For example, the mouse showed inhibition of pyruvate dehydrogenase at 15 min for the 0.1 and 1.0 mg/kg doses and at 30 min for the 1.0 mg/kg dose. However, the total arsenic level in blood was evaluated for 2 hr for the 0.1 mg/kg dose and for 4 hr for the 1.0 mg/kg dose. It is postulated that the form of arsenic in the blood is the important factor and additional experiments are planned to test this hypothesis.

Gas particles have been prepared for the experiment designed to determine if GaAs causes fibrosis up to 6 months after a single intratracheal dose of 100 mg/kg. The preparation of these particles has been a problem that is still under investigation.

Significance

Despite the fact that most investigators do not feel that the rat is an appropriate model for arsenic toxicity, there has been little comparative toxicity data to choose another species. We now have the data and can pick another species based on scientific evidence. We can proceed to the GaAs experiments with the knowledge that we can determine systemic arsenic toxicity.

Publications - None to date

TANNIN AS A BYSSINOTIC AGENT-EFFECT ON ENDOTHELIAL CELLS

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Occupational Lung Disease
1 R01 OH02116-01
06/01/85 - 05/31/88
$60,105 ($60,105 Cum)

Objectives

Byssinosis is a significant occupational health problem among cotton mill workers. The source of the byssinotic agent appears to be the cotton bract, although the specific causative bract component or components are unknown. This project comprises an evaluation of the effect of condensed tannin, a purified component of the cotton bract, on vascular endothelial cells. The overall goal of this proposal is to characterize the effects on vascular endothelial cells medicated both by purified tannin and by aqueous extracts of the cotton bracts from which the tannin was isolated. We believe that an understanding of the cellular toxicity of tannin may offer insights into the pathophysiology of byssinosis.

Methodology

Specific Aim 1: Characterization of time and dose dependencies of endothelial cellular toxicity mediated by tannin and bracts extracts.

The experiments addressing this specific aim consist of assays of cellular toxicity using ^{51}Cr release from endothelial cells exposed to varying doses of tannin for varying periods of time. These experiments yield toxicity curves of tannin effects on the endothelial cells.

Specific Aim 2: Determination if tannin alters paracellular endothelial permeability prior to the onset of cell death.

Experiments in this specific aim are intended to determine if the barrier function of endothelial cells is affected by tannin, that is, if an endothelial monolayer might be affected by tannin in other ways than simple cell death. The methods to accomplish this aim involve measurement of paracellular permeability using exclusion of vital dye by endothelial cell monolayers.

Specific Aim 3: Determination if tannin promotes detectable changes in endothelial cell structure or function prior to cell death.

The experiments in this specific aim will determine if significant sublethal injury is induced in the endothelial cells by tannin, and whether such injury significantly affects cellular function. These experiments assess several parameters of cellular function, including cellular protein synthesis, serotonin uptake, and pinocytotic rate.

Specific Aim 4: Characterization of the effects of chronic, sublethal exposure to tannin on endothelial cell function.

This last specific aim builds upon the data accumulated in the first three specific aims. We will determine the minimum tannin dose and extent of exposure required to induce the various specific toxic effects delineated in the first three specific aims. This is of major importance in regard to cumulative toxicity that a cottom mill worker could experience over a many year exposure to cotton bracts. An important aspect of these experiments is the reversibility of any of the observed tannin-induced effects described above.

Progress & Accomplishments

Specific Aim 1: The experiments proposed in Specific Aim 1 have been accomplished and are described in the publication listed below. We have defined the time and dose dependency of tannin toxicity to endothelial cells, and compared these data to tannin effects on peripheral skin fibroblasts. These data show that the endothelial cell appears to be particularly, perhaps uniquely, susceptible to tannin-mediated effects.

Specific Aim 2: We have not as yet performed experiments that address paracellular endothelial permeability, although we have now succeeded in consistently growing the endothelial cells on the cytodex beads. Therefore we expect that these experiments of Evan's blue dye inclusion can be accomplished in the next six to nine months.

Specific Aim 3: We have performed experiments of cellular protein synthesis during the initial, prelethal, period of exposure to tannin as described in the grant proposal. Although cellular release of ^{51}Cr does not occur until after three hours of tannin exposure and protein synthesis continues for several hours following the initiation of a toxic tannin dose, synthesis of all proteins is profoundly diminished within the first half hour of exposure. We have determined this using endogenous labeling experiments of serial pulses of ^{35}S-methionine in tannin exposed cells. We are presently performing experiments of cellular transport of various amino acids to determine if this toxicity is mediated by, among other things, an interference with transmembrane uptake of amino acids and glucose. Our morphologic observations accomplished in Specific Aim 1 lead us to believe that the primary toxic effect of tannin is on cellular membrane function.

Significance

We believe that the major significance of the project so far is in describing the dose and time dependencies of endothelial cellular toxicity to cotton bract tannin. We find it of great interest that skin fibroblasts, a mesenchymal cell remote from the vascular tree, is relatively resistant to tannin-mediciated toxicity. Further, there are obviously profound membrane effects provoked in the endothelial cells by cotton bract tannin. In addition, we have shown that the toxicity to endothelial cells by crude cotton bract extract is primarily accounted for by the tannin in the extract. Since bract extract is a complicated mixture of materials, this observation supports our original hypothesis that tannin may be an important byssinotic agent.

Publications - Abstract(s) available

Johnson CM, Hanson MN, Rohrbach MS: Toxicity to Endothelial Cells Mediated by Cotton Bract Tannin: Potential Contribution to the Pathogenesis of Byssinosis. Amer J of Pathology 122:399-409, March 1986.

MECHANISM AND TREATMENT OF PHOSGENE POISONING

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Occupational Lung Diseases
7 R01 OH02264-01
08/01/84 - 07/31/87
$127,792 ($127,792 Cum)

Objectives

1) Characterize the pathophysiology and time course of acute oxidant lung injury from toxic gas inhalation, as represented by phosgene as a model gas.

2) Determine if agents which elevate cyclic adenosine monophosphate (c-amp) can attenuate lung injury and edema formation by preventing phagosome granular enzyme release and toxic oxygen radical generation, or by attenuating contraction of pulmonary epithelial and endothelial cytoskeletal components, thereby preventing separation of tight junctions between cells. The latter phenomenon has been suspected to contribute to edema formation in acute lung injury.

3) Determine if the delayed onset of symptomatic pulmonary edema seen clinically in toxic gas inhalation is related to lag time necessary for sufficient accumulation of lung edema, from an unquenched chain of lipid peroxidation, or from secondary influx of neutrophils, creating a second wave of endogenous inflammation. A series of experiments related to this objective will be performed to determine if parenterally available sulfhydryl scavengers such as N-acetylcysteine can attenuate lung injury from phosgene by quenching ongoing lipid peroxidation.

4) Determine if mediators of arachidonic acid metabolism are important in the pathogenesis of acute oxidant lung injury from phosgene. Experiments related to this objective will also yield information whether corticosteroids or conventionally available non-steroidal inflammatory drugs will be of benefit in preventing lung injury from phosgene.

5) On the basis of work generated toward the above objectives, formulate a therapeutic plan for treating humans exposed to toxic oxidant gases, using conventionally available pharmacologic agents. Because clinical research involving therapeutic treatment protocols would be logistically difficult and because withholding any potential useful therapy would be unethical, we would propose that humans exposed to oxidant gases be treated according to therapeutic protocols found effective in animals in this and other studies.

Methodology

Male New Zealand white rabbits will be exposed to 200 ppm phosgene in air in a specially designed exposure chamber constructed by the University of Tennessee. Duration of exposure will be varied to regulate the intensity of exposure (in ppm-min). Following exposure, rabbits will be transported to the lung perfusion laboratory, where they will be anesthetized and heparinized. The animal will be exanguinated through a left thoracotomy, and the lungs perfused through pulmonary artery and left atrial cannulas in a recirculating manner with protein free Krebs-Henseleit solution. Lungs will be ventilated via tracheostomy with 5% CO_2 in air using a Harvard respirator. Pulmonary artery, left atrial, and tracheal pressures will be measured with pressure transducers. Lung weight gain from edema formation will be monitored by suspending the circulation reservoir from a force displacement transducer. All interventions will be administered immediately after exposure to phosgene, and continued by adding sufficient concentrations of drugs to the perfusate.

Progress & Accomplishments

Difficulties were encountered in constructing a satisfactory exposure facility; however, it is now functional and initial exposures are being performed to determine a dose (ppm-min) which produces lung edema without immediate death during exposure. During Year 1, when we were unable to perform phosgene exposures, work was performed on a new antiarrhythmic drug, amiodarone, which causes pulmonary fibrosis in man in a substantial minority of patients receiving the drug. We have established that the drug produces lung toxicity by acting as an oxidant similar to paraquat. We have also demonstrated that many of the interventions planned for the phosgene experiments (N-acetylcysteine, ibuprofen, corticosteroids) prevent acute lung injury from amiodarone infused into the inflow circuit in ventilated and perfused rabbit lungs.

Significance

Based on these findings with amiodarone, we are hopeful that the sulfhydryl donor N-acetylcysteine and the non-steroidal ibuprofen will prove useful in attenuating lung edema from inhaled oxidants such as phosgene.

Publications - Abstract(s) available

LUNG COLLAGEN IN SILICOSIS: FIBROSIS MECHANISMS

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Occupational Lung Diseases
5 K01 OH00002-02
09/28/84 - 08/31/87
$32,400 ($64,800 Cum)

Objectives

There are four specific aims to be accomplished in this project:

1. To test the hypothesis that collagen crosslinking in silicotic rat lungs differs from that found in normal lungs in one or more of the following aspects: type and amount of crosslinks per collagen molecule, time course of crosslink formation, turnover of crosslinks, reducibility of crosslinks, and location of crosslinks.
2. To test the hypothesis that crosslinking in silicotic rat lungs differs from that found in bleomycin-exposed rat lungs.
3. To determine whether elastin crosslinking can be measured in the lungs of either normal adult rats or rats exposed to fibrogenic stimuli (silica, bleomycin), and if so, to determine if there are differences among the groups.
4. To develop simple screening assays of urine or blood, based on data obtained in Specific Aims #1-3. indicative of early silicotic changes.

Methodology

We have developed techniques for separation and quantification of both conventional amino acids and collagen crosslinks using a flexible reverse-phase HPLC system. The equipment, reagents, and elution programs used for such analyses have been described by us in detail (Reiser and Last, Liquid Chromatography 1:498-502, 1983). Briefly, C_{18}RP columns of varying dimensions are used in conjunction with either isocratic or gradient elution systems using SDS and n-propanol in phosphate buffers. Reducible difunctional crosslinks are detected both by incorporated radioactivity following reduction with NaB^3H_4 and by post-column derivatization with o-phthalaldehyde. The trifunctional nonreducible crosslink hydroxypyridinium is detected by its natural fluorescence. The elastic-specific crosslinks, desmosine and isodesmosine, are detected by measuring absorbance at 275 nm. Elastin crosslinks are analyzed in tissues subjected to a modified Lansing procedure.

Silicosis will be induced in rats by intratracheal instillation of 50 mg of crystalline silica, a technique used successfully in previous studies. Fibrosis will also be induced by intratracheal instillation of 1-1.5 u of bleomycin. In vivo labeling of crosslinks will be accomplished by implanting Alzet minipumps containing 800 uCi (^3H) lysine. Pilot experiments have demonstrated that even crosslinks in adult rat lungs will incorporate label using this approach.

Crosslink excretion in urine will be analyzed in control and fibrotic rats. Pilot studies have demonstrated that there are sufficient crosslinks for analysis in the urine produced by one rat over 4-5 days. Rats will be kept in metabolic cages during the week following pump implantation to collect labeled urine. Urine is prepared for analysis by acetone precipitation, followed by reduction and hydrolysis.

Progress & Accomplishments

During the first year of this project, we optimized in vivo labelling techniques and concluded that implantation of minipumps containing isotope was the most efficient way of labeling crosslinks in adult rat lungs. Once this labeling protocol was optimized we were able to begin instilling silica and implanting minipumps in the rats. This phase of the project has gone very smoothly so far with virtually no deaths. Tissue is currently being collected and prepared for analysis. We completed studies in which we examined difunctional crosslinks and hydroxypyridinium in silicotic rat lungs available from an earlier study. Time points ranged from 1 week to 1 year. We found striking differences between silicotic and control lungs. These findings have been reported in a manuscript accepted for publication pending minor revisions. We have also examined crosslinks in bleomycin-induced fibrosis and again found significant differences from controls. These findings have been reported in papers in press. We optimized a protocol for analyzing crosslinks in urine; preliminary data suggest that crosslink excretion may be different in silicotic rats. These techniques will be used to analyze the urine from the in vivo labeled rats.

Significance

Silicosis has been one of the most studied of the pulmonary fibrotic disorders, yet the underlying mechanisms remain obscure and early diagnosis of exposed workers is extremely difficult. Consequently, animal models have been used to investigate a wide range of histochemical, morphological and biochemical changes. In previous studies, we have observed that experimental silicosis differs in many respects from other animal models of pulmonary fibrosis induced with agents such as paraquat, ozone and bleomycin. For example, the percentage of protein synthesis committed to collagen synthesis does not increase in silicosis, nor is there an increase in collagen type I:type III ratios as occurs in other models. In short, lung collagen appears more "normal" metabolically in silicosis than in other types of fibrosis. In order to investigate the metabolic derangements that cause this apparently "normal" collagen to become deposited in fibrotic nodules, we decided to analyze collagen crosslinks as a way of tracking the metabolic fate of lung collagen from tropo-collagen to mature collagen fibers. Our preliminary data thus far have suggested that site-specific alterations in the level of lysine hydroxylation in silicosis may be a key event in determining whether collagen is destined to become fibrotic. Identification of the derangement at the molecular level should provide insight into the still poorly understood mechanisms of this disease, and may lead to the development of rational strategies for both intervention and early diagnosis.

Publications - None to date

OXYGEN FREE RADICAL IN PULMONARY FIBROSIS

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University of Vermont	09/28/84 - 08/31/87
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Summary

Exposure to asbestos and silica is associated with a variety of pulmonary disorders including pulmonary interstitial fibrosis. Although asbestosis and silicosis have been recognized as occupational lung diseases for nearly a century, the mechanism(s) by which these fibrogenic minerals induce lung cell injury and fibrosis is speculative, particularly at the cellular level.

In this proposal we hypothesize that oxygen free radicals may be one important mediator of lung cell damage and/or fibrosis *in vitro* and *in vivo* after exposure to fibrogenic minerals. To test this hypothesis, we will undertake the following studies:

1. Cytotoxicity induced by exposure *in vitro* to various concentrations of asbestos fibers and silicon dioxides will be determined in lung fibroblasts, alveolar macrophages and epithelial cells.
2. Production of superoxide radical (O_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH) by these cells after exposure to minerals at various concentrations will be monitored and correlated with results of #1 above.
3. Levels of superoxide dismutase (SOD) in lung fibroblasts, alveolar macrophages and epithelial cells after exposure to minerals *in vitro* and in lung sections of rats after inhalation of asbestos *in vivo* will be measured using quantitative biochemical assays and immunocytochemical techniques.
4. We will determine whether mineral-induced injury to alveolar macrophages, fibroblasts and epithelial cells *in vitro* can be prevented by the addition of scavengers of O_2 and OH.

These studies will be important in elucidating the importance of oxygen free radicals in mineral-induced lung cell injury, asbestosis and silicosis. In addition, they will contribute information on possible preventative and therapeutic approaches to these serious occupational diseases.

Publications - None to date

SILICOSIS: IMMUNOLOGICAL ABNORMALITIES

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Occupational Lung Diseases
5 K01 OH00018-02
09/28/84 - 08/31/87
$32,400 ($64,800 Cum)

Objectives

To compare immunologic findings in silicotic subjects, identified in an epidemiologic study of respiratory disease among Leadville, Colorado residents, with those in community controls and in nonsilicotic miners having substantial past silica exposure.

Methodology

Silicotics and two appropriate comparison subjects matched for age within five years will be interviewed by a local public health nurse; will receive intradermal tests for delayed hypersensitivity to PPD, tetanus toxoid, and candida antigens; and will give a blood specimen for complete blood and differential counts, immunoglobulins, complement, immune complexes, autoantibodies, and separation of mononuclear cells. Detailed immunologic studies on peripheral blood mononuclear cells include lymphocyte proliferation to three mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and to three antigens (purified protein derivative, candida, and tetanus toxoid); cells, suppressor cells, and natural killer cell phenotypes; lymphokine production, assayed for interleukin-2 and interferon; and evaluation of natural killer cell activity. Immunologic differences found for silicotic subjects in comparison to dust exposed and unexposed controls will be related to profusion of small and large opacites on chest radiographs and to the presence of mycobacterial infection due to M tuberculosis or atypical mycobacteria. Silicotics wishing to have bronchoalveolar lavage in an independent study will have constituents of lavage fluid compared to T-cell phenotypes in peripheral blood.

Progress & Accomplishments

Preparation for the field portion of the study is underway.

Significance

Preliminary data suggest that silicotic subjects, in comparison to their wives, have decreased percentages of helper T-cells and increased percentages of lymphocytes of the natural killer cell phenotype. Through this study effort, we will seek to identify immunologic factors which may predispose dust-exposed individuals to silicosis and which may predispose silicotic subjects to mycobacterial infections. Further understanding of the immunology of silicosis may suggest primary and secondary prevention measures which can be confirmed in future prospective studies.

Publications - None to date

EFFECT OF MINERAL EXPOSURE ON MACROPHAGE FUNCTION

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Occupational Lung Diseases
5 K01 OH00019-02
09/28/84 - 08/31/87
$27,801 ($59,737 Cum)

Objectives

The effects of in vitro and in vivo (inhalation) exposure to minerals found in association with coal mine dust on function of individual alveolar macrophages will be studied. Comparison between in vitro and in vivo measurements will permit evaluation of the feasibility of using these in vitro techniques to predict in vivo effects.

Methodology

Dose-response and particle size dependent studies on functional alterations will be conducted using exposure levels which are sublethal to the cells. Two separate techniques will be used to evaluate toxic effects on cells lavaged from male Long Evans hooded rats. Electrophysiological voltage clamp experiments will be used to determine the effect of exposure on plasma membrane permeability to ions and electro-optical techniques will be used to measure the effect on production of superoxide anion, an antibacterial agent produced by the cell. In order to correlate functional alteration with exposure to minerals, backscattered electron imaging and X-ray microanalysis will be performed on individual cells to identify those cells which have phagocytized the materials.

Progress & Accomplishments

An electro-optical method has been developed to measure superoxide (SO) release from single pulmonary alveolar macrophages (PAM) collected from lungs of rat by tracheal lavage. PAM were washed and resuspended and aliquots placed in HEPES buffer in a culture dish (37^{o} C) on the stage of an inverted microscope. During adherence to the plate surface, cells released SO which was measured by the reduction of nitroblue tetrazolium (NBT), contained in the medium, to a formazan percipitate.

Cells contained in a microscopic field were transilluminated at 550 nm (absorbance peak for formazan), televised and video recorded for forty minutes. The cells became progressively darker as the formazan percipitate was produced. Temporal changes in light intensity over the cells was measured by playing the recorded video images through a photometric analyzer. The light intensity data were converted to a relative mass of formazan produced using the Beer-Lambert law.

Formazan production was represented by a first order model with time delay indicating that PAM are self-regulating in that they reached a new steady state and stopped releasing superoxide. Heterogenity was apparent since under the same conditions different cells released different amounts of SO at different rates. Changes in light intensity were found to be due to formazan production since removal of NBT from the medium resulted in no change in light intensity over the forty minute measurement period. Several blockers of SO release were tested to determine the specificity of NBT reduction for SO release. Production of and rate of release of SO were reduced 46% to 76% demonstrating that the majority of formazan production is due to SO release. The method developed is the first to quantify SO release from single cells and should be useful for studying single cell dynamics, heterogenity and activation of superoxide producing system.

Advances in the quantification of formazan production have been made this year with the purchase of video digitization equipment and software. Additional software has been written for the computer based system which will allow automated determination of changes in optical density over cells in the video field. Use of this equipment will also minimize the distributional errors inherent in microspectrophotometry. This equipment will be in full operation in the near future.

Preliminary measurements have also been made in the electrophysiological portion of the study. Successful whole cell patch clamping has been performed and to date we have measured the resting membrane potential and stability in a number of cells. Cells have stayed attached to the electrode for up to 30 minutes and exhibit membrane potentials in the range of -55 to -80 mV.

With the success of these techniques, evaluation of the effect of *in vitro* and *in vivo* (inhalation) will begin in the next year of the grant period.

Significance

Comparison between *in vivo* and *in vitro* exposure will permit evaluation of the feasability of using these *in vitro* techniques to predict *in vivo* effects. This interdisciplinary approach should yield important information on the cellular sites of toxicity of minerals and should provide data to aid in assessing risks following inhalation.

Publications

Digregorio K, Cilento E, Lantz RC: The development of an electro-optical technique to measure superoxide release from pulmonary alveolar macrophages exposed to coal dusts. Proceedings of Coal Mine Dust Conference, Oct. 8-10, 1984, Morgantown, WV p 222-226.

GAS AND PARTICLE DEPOSITION IN TRACHEOBRONCHIAL AIRWAYS

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Occupational Lung Diseases
5 K01 OH00022-02
09/28/84 - 08/31/87
$32,400 ($64,800 Cum)

Objectives

The research is the first stage of a program focused on the development and critical evaluation of quantitative predictive models for gas and aerosol deposition efficiency in lung airways in humans and animals under a variety of respiratory modes and rates. The initial objective is to develop realistic physical models of the tracheobronchial airways of humans and experimental animals. The second objective is to use the models to examine the intrabronchial dose distribution of both gases and particles. The ultimate objective is to develop predictive models for deposition using both theory and experimentally derived coefficients, including models that account for the pronounced nonuniformity of epithelial deposition on the airways.

Methodology

Single path hollow airway casts of human and dog tracheobronchial airways will be built, including a cast composed of all major branches, a cast composed of minor branches, and a cast containing approximately equal numbers of major and minor branches. Flow distribution will be measured prior to cast pruning, and each branch removed will be replaced with an equivalent flow resistor. The casts will be fabricated so as to permit repeated assembly for experimental deposition measurements.

Aerosol deposition in the small airways will be examined for the distinctly nonuniform deposition patterns that have been previously observed in the major human airways and, more recently, at alveolar duct bifurcations in rodents. The utility of the casts for quantitative detailed gas dosimetry will be demonstrated using NO_2 as a model air pollutant and triethanolamine (TEA) as an absorbing surface. Overall deposition efficiencies at each airway segment and the pattern of deposition within airways will be defined for various constant and cyclic flow rates.

Progress & Accomplishments

Hollow airway casts which extend to 1.0 mm diameter airways are required to evaluate the flow distribution in this model system. Positive wax casts of dog airways and one human cast have been produced using new combinations of waxes to provide a mold which will be sufficiently rigid to maintain the structural integrity of the small airways while withstanding further handling. Following morphometric measurement the hollow casts will be made by a "lost wax" process. Epotuf (Reichold Chemical, Inc.), a new plastic resin, has been used for preliminary casting of small segments of dog lung. This new clear plastic is rigid and machinable. This is important for the flow measurements and for construction of a reusable single pathway cast.

Initial data obtained in a coordinate study on regional deposition on the flow division through a central cast of the human tracheobronchial tree show no significant change in the division of flow for constant inspiratory flow rates from 125 to 1000 cm^3/s. Fixed resistors may thus be satisfactory for the single pathway cast if similar results are obtained for cyclic flow.

The deposition of ultrafine aerosol particles in the larynx, trachea and major bronchi, which had been measured in the central human airway cast, was examined to determine what information may be obtained about the airflow from the deposition patterns. Total deposition in the airway cast plus larynx ranged from about 0.5% to 4.0%. Thus, the depletion of the aerosol concentration as it passes through the system may be neglected, at least initially, in calculations for model development. The measurements are consistent with deposition by diffusion being the dominant mechanism for these particles, (d = 0.2, 0.15 and 0.04 um) since total measured deposition was greater at a flow rates of 300 cm^3/s than at 600 cm^3/s for each particle size, and increased as the particle size decreased. At the higher flow rate, however, the deposition was lower for 0.15 um than for 0.2 um particles. This suggests increased deposition of 0.2 um particles by interception or impaction because of increased turbulence.

The change in the relative amounts of material deposited on the artificial larynx as compared with the airway cast as flow rate changes is informative. The amount of activity deposited in the cast exceeded that in the larynx for all three particle sizes at the lower flow rate, but at the higher flows the amount in the larynx was approximately equal to, or exceeded, that in the cast. This clearly indicates that a more significant disturbance of flow is introduced by the larynx at the higher flow rate. An overall trend of decreasing surface density is seen along the trachea which has a steeper slope at the lower flow rate. The sharper decrease seen at the lower flow rate reflects the more rapid damping of the less energetic eddies.

The overall mean Reynolds number in generations 0 and 1 of the cast in which the measurements were made ranged from about 650 to 2790. Thus, the flow will be neither fully laminar or turbulent. When the measured deposition efficiencies averaged over the trachea as a whole are compared with those predicted for diffusion from either laminar or turbulent flow the experimental values are approximately 15 times as great as those predicted for either flow regime.

Significance

The proper development of dose-response relationships for occupational respiratory diseases requires understanding of the determinants of the dose to critical respiratory tract tissues from inhaled gases and aerosols. Knowledge of the deposition patterns and efficiencies within airways is essential for the production of reliable dosimetric models. Predictions or aerosol deposition in the central airways based on uniform deposition are clearly unsatisfactory. Present models are not able to deal with the inhomogeniety of deposition demonstrated in the cast system. It is anticipated that as this work progresses, more realistic theoretical models will result from better understanding of the airflow patterns in the airways. In addition the differences and similarities between deposition patterns in the human dichotomous branching tracheobronchial tree and the canine monopodial branching will be documented. This is important if results obtained in experimental animal models are to be properly extrapolated to humans.

Publications - Abstract(s) available

BYSSINOSIS/CHRONIC BRONCHITIS IN CHINESE COTTON TEXTILE WORKERS

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Havard School of Public Health	1 R03 OH01677-01
665 Huntington Avenue	07/01/83 - 12/31/84
Boston, Massachusetts 02115	$14,071 ($14,071 Cum)

Objectives

The objective of this proposal is to study the respiratory effects of exposure to cotton dust in a population of cotton textile mill workers in Shanghi, China. The population selected for the survey processes hand-picked cotton believed to contain a lower trash content than most grades of machine-picked cotton. Since the active agent for byssinosis is thought to be contained in the trash component of cotton, this population should be exposed to less physiologically "active" dust.

Methodology

The study group was exposed to relatively low levels of inhalable cotton dust since the introduction of dust control measures approximately 20 years ago. The long history of stable exposure and range of exposures among different work areas permitted examination of exposure-effect relationships.

Two textile mill populations were selected for study: one exposed to cotton dust (n=445) and the other unexposed silk workers (n=485). A standardized questionnaire for respiratory symptoms was administered as well as before and after shift pulmonary function tests after a 2-day weekend.

Environmental sampling consisted of gravimetric dust measurements and gram negative endotoxin assays from electriated dust samples.

Request for support was directed at the analysis of the above data:

1. The prevalence of byssinosis and chronic bronchitis in cotton textile workers was examined with respect to exposures and jobs, controlling for confounders and examining interaction with cigarette smoking.
2. The prevalence of chronic bronchitis among cotton textile workers compared with silk controls.
3. Quantify the acute change in lung function and compare this to job and exposures controlling for likely confounders.
4. Examine cross-sectional lung function in the cotton textile workers for relationship to job or dust exposure, adjusting lung function using data from the silk textile workers. Analysis included multiple logistic and linear regression models.

Progress & Accomplishments

The aims as stated above were met. Analysis indicated an overall byssinosis prevalence of 7% in the cotton-exposed group versus zero in the silk controls. Chronic bronchitis occurred more frequently in the cotton workers and smoking was found to have a greater than additive effect in producing chronic bronchitis, but not byssinosis. Acute decrements in FEV1 were noted in the cotton-exposed group, and the changes were more pronounced in workers with more years of cotton dust exposure. Internal analysis of cotton-exposed workers revealed no dose-response for acute change in lung function or byssinosis prevalence, but the range of exposure was narrow (3-fold).

We clearly showed an exposure effect for symptoms and acute change in lung function. Further analysis is being done to compare airborne endotoxin and cotton dust exposure in relation to symptoms and pulmonary function.

Significance

Results are useful in that:

1. We demonstrated a significant prevalence of byssinosis and chronic bronchitis in both non-smoking and smoking textile workers exposed to low-trash content cotton at moderate levels.
2. Physiologic tests of lung function confirmed acute decrements after exposure.
3. Cohort was defined for future, prospective, study of the chronic effects of cotton dust exposure on respiratory function.

Publications - None to date

RESPIRATORY DISEASE IN BERYLLIUM WORKERS

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665 Huntington Avenue	08/01/84 - 07/31/86
Boston, Massachusetts 02115	$13,132 ($32,257 Cum)

Objectives

This project is an investigation of the respiratory effects of long-term, low-level exposure to beryllium (Be). Beryllium refinery workers have been studied and beryllium exposure data, chest x-rays, and measures of lung function were collected over a period of six years. These data are being analyzed to quantify exposure-response relationships for beryllium, while exploring techniques which may be useful for studying other occupational respiratory disease as well. The project has the following objectives:

1. The characterization of the type and degree of lung dysfunction in workers with chronic exposure to beryllium.
2. The development of exposure-response models of beryllium's effects using both continuous (pulmonary function and blood gas data) and discrete (x-ray and symptom reports data) measures of respiratory effects studied cross-sectionally and longitudinally.
3. The quantification of exposure-response relationships for beryllium exposure and pulmonary function among different population subgroups.
4. The investigation of time trends in disease development, including the possibility of the reversibility of the disease.

Methodology

Existing data are relied upon entirely for this project. The health effects data have been collected for a health monitoring program resulting from collective bargaining agreements between management and labor. The industrial hygiene data have been collected from a variety of published and unpublished sources.

Lifetime exposure histories for each worker are estimated from detailed work histories and estimates of job- and year-specific air levels of beryllium. Exposure histories are then used to estimate variety of exposure parameters for each worker, based on biological models of the disease mechanism. These exposure parameters are then used as independent variables in multivariate models of the various measures of lung dysfunction. Adjustment for confounding and effect modification is accomplished by inclusion of additional terms for such factors as smoking, age and height.

Cross-sectional models using the data from the first health survey are performed first, and investigate the relationships between each workers estimated exposure to beryllium and his/her own level of lung function as compared to that of others in the cohort. Longitudinal analyses using data from successive years of examination then can be used to examine the effects of beryllium exposure on each worker's rate of change in his/her own level of function.

Progress & Accomplishments

Progress has been made on this project consistent with the original objectives. Health examination data have been coded into a computer data base and are ready for analysis. Industrial hygiene data covering nearly all of the history of operation of the industry was collected, edited, interpreted and coded. Each job was analyzed and beryllium exposure estimates made for it for each year of plant operation. Work histories for each worker were coded. Exposure histories for each worker were calculated, and a series of 24 exposure parameters estimated for each worker. The selection of exposure parameters was based on a detailed study of the cellular mechanisms of beryllium toxicity and the development of a simplified model of these mechanisms. The exposure parameters are now ready for use in multivariate models of exposure-response, as discussed in the objectives and methodology sections.

Significance

At least 30,000 workers in the United States are exposed to beryllium. Most of these workers are widely dispersed in small factories making epidemiologic study of beryllium's health effects difficult. This project represents one of the few opportunities to follow a well-defined cohort of beryllium-exposed workers to study the chronic, sub-clinical effects of exposure to the metal.

The methods being developed in this project to estimate exposure parameters to represent lifetime exposure histories to a toxin may be useful in studies of many other occupational health hazards.

Publications - None to date

LUNG PROTEASES AND ANTIPROTEASE IN COAL DUST EXPOSURE

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Occupational Lung Diseases
5 R03 OH01715-02
05/01/84 - 04/30/86
$24,750 ($50,250 Cum)

Objectives

Extended human exposure to respirable coal mine dust is associated with the development of coal workers pneumoconiosis (CWP). Focal emphysema is a part of simple CWP. The lesion has been ascribed to contraction of connective tissue fibers deposited in this locus with consequent enlargement of adjacent airspaces. Alternatively this lesion may result from elastin lysis from elastolytic enzymes produced in either polymorphonuclear leukocytes (PMN) and/or alveolar macrophages (AM). The purpose of this proposal is to study the hypothesis that elastase secreted by alveolar macrophages following in vivo exposure of laboratory animals to bituminous coal will create focal emphysema and simple CWP. The ability of macrophages which have phagocytized coal dust to produce and secrete enzymes in concentrations which exceed the capacity of natural protease inhibitors α2-macroglobulin (α2-M) and alveolar α1-protease inhibitors will be studied to evaluate the role of coal on lung protease anti-protease balance.

Methodology

Bituminous coal of known composition was processed by differential centrifugation in sucrose solution to remove large particulate matters (20u) and to minimize submicronic particles. Coal suspensions in quantities of 2 to 4 mg in 0.2 ml saline were introduced intratracheally into male Syrian Golden hamsters. Control hamsters each receives a similar volume of sterile saline. The inflammatory response of the lung following a single intratracheal (IT) instillation of coal was studied by bronchoalveolar lavage (BAL) 3 days after instillation. Total and differential cell counts were performed on each of the hamster BAL fluid following centrifugation. Total elastolytic potential of the lavaged leukocytes and alveolar macrophages were determined by elastolytic measurements using a radiolabelled soluble elastin substrate. The α_1-PI and α_2-M levels in the BAL fluid were determined by radioimmunoassays (RIA) using specific anti-sera developed for each of the purified hamster α_1-PI and α_2-M proteins. The functional activities of the α_1-PI and α_2-M in the BAL fluid were also measured. These parameters were compared and related stoichiometrically to determine the effect of coal instillation on the protease and anti-protease balance within the alveolar environment of the coal hamsters and the saline controls. The effect of multiple exposure to coal particles on the protease and anti-protease balance was also performed by repeated IT instillations of 40-62 mg of coal at 5-7 day intervals over a 4-5 week period. The stiochiometric balance of the BAL fluid protease and anti-protease were similarly determined at 3 days and at 90 days after the final instillation. These biochemical parameters were compared with the pathological studies including histological evaluation of hematoxylin and eosin, trichrom, elastin, reticulum and connective tissue stains on the corresponding lung sections of the coal and control hamsters.

Progress & Accomplishments

In the single instillation studies, the alveolar macrophages in the alveolar fluid (AF) were cultured and the conditioned media (CM) analyzed for elastolytic activity. α_1-PI and α_2-M plasma values were also determined. The total cells, AMs and PMNs in the AF of hamsters receiving 4mg of coal were significantly greater than that of controls whereas in the group receiving 2mg only the PMN showed significant increase. Elastase activity in the CM of cultured AM was increased in animals receiving both 2 and 4 mg of coal (Control: 59.0 ± 10.5ng; 2mg: 62.6 ± 7.6ng; 4mg: 81.1 ± 12.9ng per animal). However, the only significant change was noted in the 4mg group. This increase in elastolytic activity in the conditioned media appears to correlate with the number of AMs in the AF. No significant alterations in elastolytic activity of AF of 2 or 4mg coal groups were observed. The total concentration of α_1-PI and α_2-M of the AF and plasma as well as their functional activity did not differ significantly between control, 2mg and 4mg coal groups. These data suggests the potential for increased secretion of elastolytic activity by the AM exposed to coal dust was enhanced without a concomitant alteration in AF concentrations of α_1-PI or α_2-M.

With multiple coal exposure, the alveolar fluid PMN was increased on day 3 ($12.7 \pm 4.1 \times 10^6$ vs $0.11 \pm 0.04 \times 10^6$ in controls (C) and declined to $5.1 \pm 0.8 \times 10^6$ at day 90. The alveolar macrophages were greatest at day 3 ($22.7 \pm 0.8 \times 10^6$) and decreased at day 90 ($13.2 \pm 1.3 \times 10^6$, control $5.1 \pm 0.3 \times 10^6$). The total elastolytic activity of the leukocytes was greatest on day 3 (97 ± 45ng) and decreased on day 90 to 35 ± 12ng, control 0. Plasma values of α_1 protease inhibitor (α_1PI) and α_2 macroglobulin (α_2M) in coal dusted hamsters were not significantly different from control at day 3 and 90. However, the AF α_1-PI and α_2-M values were significantly greater than controls at 3 and 90 days. The Elastase Inhibitory Capacity (EIC) of the AF at both periods coal was significantly greater than control but the AF specific activity (SA) was not altered. These data indicate a simultaneous increase in the elastolytic burden and the protease inhibitor activity of the AF following coal dust instillation. Emphysema is not observed histologically indicating the dominance of the antiprotease response in the protease-antiprotease balance.

Significance

The present proposal is directed specifically to the study of coal dust on the level of proteases and anti-proteases found in the lung. While pathological changes in coal dust exposed animal lungs have been documented, biochemical changes on proteolytic activities in response to long term coal dust challenges has never been reported. If the overall amount of PMN or AM elastase is increased in coal dust exposed hamsters, one would assume that proteolytic damage is an important determinant for coal induced emphysema, provided that the level of antiproteases (in the lavage fluid) does not change. A decreased anti-proteolytic activity will, by similar interpretation, also produce an emphysema-like lesion even if the proteolytic destruction of lung connective tissue is not augmented. This study will provide needed information on the role of the protease anti-protease balance in the onset and progression of CWP and may lead us to a better understanding of the development of coal workers pneumoconiosis.

Publications - Abstract(s) available

FOLLOW-UP STUDY OF RESPIRATORY DISEASE IN FIREFIGHTERS

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School of Hygiene and Public Health	5 R03 OH01756-02
Johns Hopkins University	07/01/83 - 06/30/85
615 North Wolfe Street	$7,985 ($25,604 cum)
Baltimore, Maryland 21205	

Objectives

The objective of the study was to evaluate the chronic respiratory effects of on-the-job exposures among firefighters. Specifically, the aims of the study were: (a) determine the annual rate of ventilatory function change measured by force vital capacity (FVC) and forced expiratory volume in one second (FEV_1), (b) evaluate change in the occurrence of chronic respiratory symptoms, including persistent cough, persistent phlegm, wheeze, and breathlessness, (c) identify occupational factors that are associated with ventilatory function and respiratory symptom changes, and (d) determine whether withdrawn from firefighting exposure is related to health.

Methodology

A follow-up study of 963 professional firefighters in Baltimore, Maryland, who participated in a health survey in the mid-1970's (baseline study) was undertaken. Information about sociodemiographic characteristics, respiratory illnesses, and occupational exposures was obtained from a self-administered questionnaire for all participants. Respiratory symptoms and smoking habits were ascertained in an interview with the British Medical Research Council respiratory questionnaire. Work history data was obtained from personnel records of the Baltimore City Fire Department. Historical records of firefighting activity were also made available by the Fire Department. Ventilatory function was assessed with spirometry.

Frequency at the baseline and follow-up studies, as well as attack and remission rates during the follow-up period, were determined for the respiratory symptoms. Rates of ventilatory function loss over the follow-up period were calculated. Crude rates were examined for selected personal and occupational characteristics. The effects of occupational characteristics identified as potential risk factors were assessed using multiple linear regression models that included confounding personal characteristics.

Progress & Accomplishments

The project has been completed. Six hundred ninety-six firefighters participated in the follow-up study, representing 71 percent of the baseline study group. The follow-up participants included 524 current firefighters and 154 men who left the Fire Department during the follow-up period. Spirograms were obtained from 628 subjects.

The remission rates were higher than the attack rates for all respiratory symptoms and the frequency of all symptoms except breathlessness fell over the follow-up period. The follow-up frequency was 12 to 13 percent for persistent cough, persistent phlegm, and wheeze and 31 percent for breathlessness. Cigarette smoking was associated with elevated rates for all symptoms. In general, blacks had lower rates than whites, though the differences were reduced after adjustment for smoking and occupational exposures. Other personal characteristics associated with the symptoms were education (persistent cough, breathlessness), age (breathlessness), and a childhood history of respiratory illness (wheeze).

The frequencies of persistent cough and persistent phlegm at the baseline study were elevated by ammonia exposure, particularly among cigarette smokers. After the baseline study, benefits from the use of respiratory protective equipment were evident. Follow-up frequencies and attack rates for persistent cough and persistent phlegm were lower among firefighters who used respiratory protective devices during the overhauling phases of fire suppression compared to men who did not use this equipment. After the baseline study, the rates of persistent phlegm and breathlessness increased with increasing firefighting activity.

Mean annual rates of ventilatory function loss were 21 ml/yr for FVC and 30 ml/yr for FEV_1. Repeatability of spirograms was assessed according to the American Thoracic Society recommendations. Firefighters with repeatable spirograms had slightly lower rates of ventilatory function loss than firefighters with nonrepeatable spirograms. Patterns of the relationships between occupational exposures and rates of ventilatory function decline were similar for men with repeatable and nonrepeatable spirograms.

Cigarette smoking was the most important factor associated with loss of ventilatory function. Other personal characteristics related to ventilatory function were age (FVC,FEV_1), weight (FVC), and blood type (FVC). Rates of decline for FVC and FEV_1 were lower among men who used respiratory protective devices while extinguishing a fire than among men who did not use this equipment.

Withdrawal from firefighting exposures through either movement to job assignments away from exposures or departure from the Fire Department was not related to the presence of respiratory symptoms or to the level of ventilatory function at the baseline study. Movement away from firefighting exposures occurred with increasing age. Departure from the Fire Department was related to evidence of heart disease.

Significance

The results of this study, in conjuction with other studies of respiratory disease among firefighters, should be reassuring to individuals in the fire service. It appears that firefighters, as a group, are not at excess risk for development of chronic lung disease. The findings, however, are not inconsistent with the development of chronic lung disease in an individual worker because of an occupational exposure. Harmful effects of combustion by-products on the respiratory tract were evident in this study. Some effects, for example, persistent cough and phlegm associated with ammonia exposures, were short-term, while the effects of more generalized firefighting exposure appeared to be long-term. Benefits from the use of self-contained breathing apparatus were evident in this study. The availability and increased use of respiratory protective devices helps to protect firefighters from developing disabling airways obstruction. In addition, the withdrawl from firefighting-exposed jobs that occurs with increasing age contributes to the absence of chronic respiratory disease.

Publications - None to date

AERODYNAMIC BEHAVIOR OF PLATELET AEROSOLS

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Occupational Lung Diseases
1 R03 OH01850-01
07/01/83 - 03/31/85
$6,445 ($6,445 cum)

Objectives

The research effort was designed to evaluate the behavior of platelet particles in viscous flow through fluid dynamic modeling, computer simulation and with actual settling experiments on micrometer-sized orthotropic aerosols. This study represents a comprehensive approach to elucidating the aerodynamic characteristics of a class of particle shapes about which little is known. The results of this research are suitable for incorporation in new or existing aerosol lung models.

Methodology

Non-spherical, orthotripic particles settling in air were modeled under dynamically similar conditions using aluminum prisms settling in viscous oil. Twenty-five rectangular prisms, each with a different length:width:thickness ratio, were settled in each of their three primary orientations and the resulting drag forces determined. Linear regression using predictors related to prism geometry and orientation was carried out to find those variables having the greatest influence on particle drag.

In a second experiment tungstic acid crystals were grown in the form of small platelet aerosol particles and the aerodynamic properties of these aerosols were compared to aerodynamic properties of polystyrene latex spheres using a spiral centrifuge. Results from this study were used to validate the results from the modeling study.

Progress & Accomplishments

At this time all work planned for this project has been accomplished. An empirical drag equation was formulated which provides extremely good estimates of the drag on rectangular prisms and other orthotropic bodies settling in air under vicous conditions. The shape resistance factors obtained using the equations are in excellent agreement with those observed by other investigators.

Significance

The potential for injury to lung and other body tissues associated with inhalation of aerosols has long been recognized. Inhaled and deposited particles, either due to their own properties or those of substances they carry have been implicated in many deleterious effects ranging from mild irritation of the nasal mucosa to long-term disability or even death. The location of particle deposits within the respiratory tract and the retention time at these sites affects the type and degree of injury.

Mathematical models have been developed to predict the patterns of aerosol deposition in the lung. Until the development of the Harris and Fraser model for deposition of fibrous aerosols, only spherical particles (or particles that behave like spheres) could be evaluated for their deposition characteristics. Due to the lack of theoretical information for the motion of non-spherical, non-cylindrical particles in the viscous flow regime which is seen in the deep portions of the lung, models have not been developed for deposition of these types of particle shape. This work has provided data to assist in the elucidation of the aerodynamic behavior of non-spherical, non-cylindrical particles.

Publications - None to date

LUNG PARENCHYMAL INJURY INDUCED BY ENVIRONMENTAL AGENTS

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Occupational Lung Diseases
5 R03 OH01856-02
07/01/83 - 06/30/85
$20,700 ($39,522 Cum)

Objectives

The goals of the study were three-fold: (1) to determine the lowest doses of toxins - bleomycin, paraquat, silica, or asbestos which induce lung parenchymal injury; (2) to determine if factors chemotactic for granulocyte and other effector cells are elaborated by lung cells exposed to these toxins; and (3) to determine the lung cell types most liable to injury from these toxins.

Methodology

Initial studies with bleomycin used a rat lung explant model. The studies were continued using single cell lines (lung fibroblasts and endothelial cells) grown in culture (1). Studies of silica exposure used human alveolar macrophages in culture (2).

Progress & Accomplishments

(1) Injury from Bleomycin

As noted in the progress report of 1984, we have shown that bleomycin in doses equivalent to peak serum levels in humans injured whole lung explant tissue in a dose dependent manner. Supernatants of explants exposed to bleomycinexhibited chemotactic activity for granulocytes. The bleomycin exposed tissues, but not their supernatants, induced granulocytes to produce superoxide anion. We conclude that therapeutic doses of bleomycin can directly injure lung tissue and that granulocytes may be attracted to lung tissue injured by bleomycin. The granulocytes may then play a role in bleomycin induced lung injury.

In further studies, we have found that endothelial cells and type II pneumonocytes are most susceptible to direct bleomycin induced lung injury. At the same time, fibroblasts are relatively resistant to the effect of bleomycin. Related studies indicate that bleomycin, at very low doses, actually augments fibroblast growth in response to known growth factors such as platelet derived growth factor. This finding may explain, in part, the exuberant proliferation of fibroblasts following exposure to agents such as bleomycin or paraquat.

(2) Silica Induced Immunoglobulin Productions

We have evaluated the interaction of silica with various concentrations of alveolar macrophages in culture. The studies have established that silica does not interfere with antigen or mitogen presentation by macrophages but does enhance macrophage interleukin-1 release. Silica also reduces the suppressive effects mediated by macrophages on blood mononuclear cells in culture, resulting in heightened immuni responsiveness.

Significance

These studies have given us further understanding of the role of immune effector cells in the pathogenesis of lung disease caused by silica and by oxidant-producing toxicants, such as bleomycin. Further, the important differences between lung cell types to injury from these agents suggests new avenues of research. These areas of research involve the molecular basis of fibrosis and fibroblast proliferation in response to these toxicants.

Publications

Moseley PL, Hunninghake GW: Bleomycin-exposed lung tissue releases chemotactic activity for neutrophils. Am Rev Respir Dis 127:56, 1983.

Moseley PL, Shasby DM, Hunninghake GW: Bleomycin induced lung parenchymal injury. Am Rev Respir Dis 130:1082-1086, 1984.

Moseley PL, Hemken C, Hunninghake GW: Bleomycin augmentation of fibroblast proliferation. Clin Res 33:581A, 1985.

Moseley PL, Hemken C, Hunninghake GW: Augmentation of platelet induced fibroblast proliferation by bleomycin. Am Rev Respir Dis 131:368A, 1985.

RESPIRATORY ABNORMALITIES OF HARDROCK MINERS

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Occupational Lung Diseases
5 R03 OH01865-02
09/01/83 - 08/31/85
$22,965 ($45,766 Cum)

Objectives

To perform a population based study of a random sample of male residents of Leadville, Colorado, wherein we will compare miners and nonminers for prevalence of chronic bronchitis and other respiratory symptoms, measurements of subclinical small airways abnormalities, and measurements of obstructive and restrictive lung disease, controlling for smoking status. Any health abnormalities found for miners will be related to indices of dust exposure.

Methodology

Subjects were invited to participate, on the basis of randomly-chosen block of residence, by a local outreach worker who conducted a census of eligible persons, aged 25 through 64 years. An interviewer recorded respiratory, smoking and occupational histories with a questionnaire. Physiologic evaluation included single breath nitrogen test (closing capacity, closing volume, and slope of the alveolar plateau), diffusing capacity of the lung, and plethysmographic measurements (total gas volume, airways resistance, total lung capacity, including forced expiratory volume at 1 second, peak expiratory flow, flow rates at 25, 50, and 75% of the vital capacity, and maximal mid-expiratory flow rate). Quality assurance for questionnaire and physiological data was based on retesting a 10% random sample. Work histories from miner subjects were spot-checked against Climax Molybdenum Company personnel records and used to calculate an index of occupational dust exposure from industrial hygiene measurements for job classifications and work locations. Data analysis was based, in part, on prediction equations for normal values of pulmonary function tests at 3100 meters, developed from data for asymptomatic nonsmokers.

Progress & Accomplishments

Field collection of data is complete. Analysis is in progress.

Significance

Through this study we hope to:

1) clarify the physiology and health implications of industrial bronchitis for hardrock miners with silica exposure.
2) assess whether symptom prevalence and pulmonary function abnormalities seem out of proportion for a miner group at high altitude where minute ventilation is increased, and
3) evaluate the field use of sophisticated pulmonary physiology evaluation, such as plethysmography and single breath nitrogen tests, for occupational epidemiology.

Publications - None to date

INTERACTION OF QUARTZ WITH BIOLOGICAL MEMBRANES

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Occupational Lung Diseases
5 R03 OH01905-02
07/01/84 - 06/30/86
$10,720 ($10,720 Cum)

Objectives

The major objective of this project is to further the understanding of the molecular nature of the interaction between the surface of biologically active minerals and cell membranes.

Methodology

Two bioassays widely used in pneumoconiosis research are hemolysis and macrophage cytotoxicity. In the hemolytic model the mineral's ability to lyse the erythrocyte membrane is determined quantitatively. The mature erythrocyte is a non-nucleated biconcave disk which contains hemoglobin. Many viologically activity minerals have the ability to bind to the membrane surface and alter the permeability of the membrane. The hemoglobin released from the cell is determined using optical spectroscopy after the intact cells, mineral dust and cell stroma are centrifuged down. A dose response curve is determined showing percentage hemolysis as a function of mineral concentration. The lower part of the curve is linear and from the equation of the line the concentration of mineral dust (usually given in mg/ml) required to lyse 50% of the erythrocytes is calculated. The value, called the HC50, is an index of the activity of a given specimen.

Erythrocytes are not known to have any role in the pathogenesis of any of the pneumoconiotic diseases. Although membrane damage to the macrophages is thought to play a central role in silicosis and asbestosis. Macrophage viability is usually determined using a staining technique and an enzyme assay. One is a morphological criteria and the other is biomedical. In our preliminary work we have used a morphological end point. Unstimulated peritoneal macrophages taken from mice have been used. The peritoneal exudate cells are allowed to adhere for one hour and the medial is then changed in a way as to remove the non-adherent cells. By definition the adherent cells are macrophages.

After twenty-four hours various concentrations of quartz are added to the adherent macrophage cultures. A flourescent staining technique has been used to determine viability. At various times after the quartz and macrophases have been allowed to interact, the media is removed and fresh protein-free media is added which contains fluorecein diacetate. The fluorecein diacetate is taken up by the macrophages which contain within the cytoplasm non-specific esterases which cleave the acetate groups.

The fluorescein is then within the cell and can be excited by ultraviolet light to fluoresce. The viable cells fluoresce while the nonviable cells are unable to cleave the ester and do not. Whether the nonviable cells take up the fluorescein diacetate and are unable to cleave the ester or are unable to take it up has not been reported.

Progress & Accomplishments

Several quartz specimens of different varieties and from different geological locations were collected and size fractionated on the basis of Stokes' settling diameter. The actual size distribution of each Stokes' diameter was then determined by transmission electron or light optical microscope depend on the actual size distribution of the particles. The hemolytic activity of each specimen was determined as a function of size distribution. The hemolytic activity of the different quartz varieties were compared holding the size distribution constant. The surface properties of quartz specimens of similar particle size were modified by treatment with acid, base and trimethylchlorosilane. The effect of these different modifications on the hemolytic activity of the mineral was determined.

Significance

The hemolytic activity of quartz closely follows the surface area of the mineral. Although the surface can exist in a number of States of vastly different activity. It is possible to have quartz which is inactive in a hemolytic model. The studies have been extended to the polymorphs of titanium dioxide (rutile and anatase) and the same concept of surface structure which was found to be important for quartz hold for the titanium dioxides.

The studies are now being extended Macrophages to determine if the same physiocochemical properties of quartz damage both erythrocytes and macrophages.

Publications

Langer AM, Nolan RP: Minerals, rocks and ore bodies: sources of agents in human disease. Proceedings of the Second International Congress on Applied Mineralogy. Park WC, Hausen DM, Hagni RD (eds), The Metallurgical Society of AIME, Los Angeles, p 1159-1177, 1984.

Langer AM, Nolan RP: Physicochemical properties of quartz controlling biological activity. In: Silica, Silicosis, and Cancer: Controversy in Occupational Medicine. Goldsmith DF, Winn DM, Shy CM (eds), Praeger Publishers; New York, p 125-135, 1985.

Langer AM, Nolan RP: Physicochemical properties of minerals relevant to biological activities: state of the art. In Vitro Effects of Mineral Dusts. Third International Workshop. Beck EG, Bignon J (eds). Springer-Verlug, Berlin, 9-24, 1985.

Nolan RP Langer AM: Particle Size and chemically-induced variability in the membrolytic activity of quartz: preliminary observations. In Vitro Effects of Mineral Dusts. Third International Workshop. Beck EG, Bignon J (eds), 39-50, Springer-Verlug, Berlin, 1985.

OCCUPATIONAL HEALTH FACTORS IN FARMER'S LUNG DISEASE

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Occupational Lung Diseases
1 R03 OH02098-01
04/01/85 - 03/31/87
$20,971 ($20,971 Cum.)

Objectives

1. Assessment of the association between circulating antibody status and the incidence of farmer's lung disease, the prevalence of respiratory symptoms, and decline in lung function with time.
2. Assessment of the association between environmental, economic and education factors and circulating antibody status.

Methodology

This study is a prospective epidemiologic analysis of a population-based random sample of 1444 farming individuals living in central Wisconsin. One hundred and forty-seven subjects demonstrated positive precipitins by double-immunodiffusion assay in 1977. In the follow-up study, these precipitin-positive subjects were matched by age, sex, and smoking status to 147 subjects who were precipitin-negative in 1977. The American Thoracic Society standardized respiratory questionnaire and pulmonary function tests were administered on participating subjects to determine if precipitin-positive subjects were experiencing more respiratory difficulties than precipitin-negative individuals seven years post-exposure. Additionally, sera were collected from all subjects to determine their current antibody status. Statistical analyses will be performed using SAS.

Progress & Accomplishments

In the first year of the study, the interview process and data collection phase was completed. Ninety-one percent response and 78% participation levels were achieved. Additionally, enzyme immunoassays have been performed on the current sera by Dr. Marx and his technical staff at the Marshfield Medical Foundation. These data have been entered into the computer and "cleaned". Preliminary analyses of these data were reported at the International Symposium on Health and Safety in Agriculture held in Saskatoon, Saskatchewan, October 8-11, 1985.

Plans for the second year of the study involve: 1) completion of the 1977 enzyme immunoassays which will then be incorporated into the master data base; 2) more detailed statistical analyses of the master data base in order to examine risk factors and trends; 3) reporting of findings to scientific journals and at scientific meetings. Publications currently in preparation include: 1) preliminary results, which was reported in Saskatoon and which will appear in the collection of papers presented at the conference; 2) "The Prognostic Significance of Farmer's Lung Disease Antibodies" - a general paper reporting the major results of the study which will be submitted to the American Review of Respiratory Disease; 3) "Occupational Practices of Female Farmers" - descriptive data which is currently non-existent and which will be submitted to the APHA Journal; 4) "Sources of Occupational Health Information for the Agricultural Community" - more descriptive data which will help health educators in rural areas focus their efforts.

Significance

A test for the presence of antibodies to farmer's lung disease antigen's is a commonly used clinical procedure and a positive result usually denotes recent exposure to thermophilic actinomycets. A population-based prospective analysis of outcome of farmers who have antibodies to farmer's lung antigens has not previously been reported in the literature and, therefore, it is not known whether farmers with antibodies are at increased risk for developing symptoms of interstitial lung disease relative to farmers without antibodies. Complicating this question is the phenomena of pulmonary mycotoxicosis, a syndrome which mimics acute farmer's lung disease with the exceptions that individuals who suffer from pulmonary mycotoxicosis do not have farmer's lung disease antibodies and that it is believed that pulmonary mycotoxicosis does not progress to chronic interstitial lung disease. The role of antibodies in the risk for chronic farmer's lung disease becomes, therefore, an important issue from both diagnostic and pathologic perspectives.

Publications - Abstract(s) available

ETIOLOGICAL FACTORS IN INDUSTRIAL LOW BACK INJURY

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Musculoskeletal Injuries
5 R01 OH00982-03
03/01/82 - 02/28/86
$30,000 ($458,110 Cum)

Objectives

1. To identify premorbid individual physical, demographic, and psychosocial factors associated with industrial back injuries and time loss.

2. To determine whether the perceived or measured physical demands of an individual's job relate to reported back injuries.

3. To determine whether physical exam findings obtained immediately following reported back injuries relate to resultant time loss.

4. To study the reliability and validity of various techniques, such as ultrasonography of the spinal canal and isometric strength testing, which have been proposed for use in identifying individuals at greatest risk for back injury.

5. To assess the validity of retrospective approaches by comparing the prospective data from this study with data generated during a previous retrospective study of the same plant.

Methodology

Blue collar employees (n=3020) of a Northwest aircraft manufacturing plant volunteered to take part in the study. The volunteers underwent cardiovascular risk screening, and a physical exam which included anthropometric measurements. lifting strength, submaximal treadmill testing, ultrasonography of the lumbar spinal canal, reflex testing, and assessment of posture and flexibility. The volunteers were also given demographic, social, and psychological questionnaires to complete. The questionnaires requested information pertaining to: past medical history, past employment history, education level, marital status, family support systems, social support at the worksite, perceptions of job demands, expectancies regarding the locus of control of physical health and well being, exposure to vibration due to riding in motor vehicles to, from, and during work, and frequency of social activities and activities of daily living. The Minnesota Multiphasic Personality Inventory (MMPI) was also included.

Job analysis was conducted so that the influence of loads on the spine imposed by various job tasks could be studied with regard to their relationship to back injuries. More than 4,000 individuals were employed at the plant during the job analysis portion of data collection. The tasks of all jobs in which 20 or more people worked were studied. Within these different jobs, the tasks involving the greatest loads on the lumbar spine were analyzed using the mathematical model developed by Schultz. The method was chosen for its ease of application and because it is the only mathematical model validated through direct measurements of spinal load as determined through intradiscal pressure measurement studies.

Over the past two years since the completion of the initial examinations, the study subjects have been tracked for reported back injuries. The onsite medical clinic staff cooperate in order that standard injury physical exam data is collected at the time volunteers report injuries. The injured volunteer is also given a questionnaire requesting similar information to that gathered premorbidly. In addition, routine injury reports are obtained, which include information relating to such things as the stated mechanism of injury and associated time loss from work.

Progress & Accomplishments

The medical screening and collection of premorbid physical exam and psychosocial data for the 3,020 study volunteers has been completed. The data collected on each subject was entered into a computer data base, and data entry errors were identified and corrected, while data ranges were evaluated. After the premorbid physical and psychosocial data collection was concluded, our efforts were directed toward the completion of the job analysis portion of the study. Presently, we have collected data on over 250 back injuries and analysis has begun for predicting acute back injury episodes.

The team evaluated the reliability of threee evaluation tools used during the collection of premorbid data: strength testing, submaximal treadmill testing, and ultrasonography of the lumbar canal. Analysis of strength testing techniques included: 1) the evaluation of loads imposed on the lumbar spine during different test lifts, and 2) statistical evaluation of testing variations. Our study led to recommendations for improving the safety of the isometric strength testing technique. Presently, we have nearly completed the data collection on 250 back injuries and analysis has begun for predicting acute back injury episodes. We have also: 1) determined the accuracy of ultrasonography for spinal canal measurements, and 2) found trends to support previous findings of smaller lumbar canal diameters in the first 100 reported back injuries. In addition, the safety of smaller lumbar canal diameters in the first 100 reported back injuries. In addition, the safety and accuracy of submaximal treadmill testing technique was established (median error 7.3% of actual VO_2 max), with the cardiovascular fitness of more than 2400 subjects measured without a complication. Our study has also illuminated the relationship of a high hyseria scale on the MMPI, smoking, obesity, lumbar canal size as measured by ultrasonography, and history of back problems requiring surgery or back treatment over the previous 10 years to subsequent injuries. Most recently, our extensive evaluation of anthropometric and flexibility data has provided the most definitive results to data regarding the controversy surrounding the importance of spinal flexibility. Flexibility relates more to aging than to back injuries. With completion of our current analyses of premorbid physical exam data with regard to back injury incidence (June 1986) we will better understand the relationship of many of the other premorbid factors to acute back injuries.

Significance

Back injuries in industrial settings result in significant time loss disability and, as such, constitute a major socioeconomic problem for most modern societies. The cost of back injuries to society and industry is enormous, making this problem the most expensive health care problem in the 30 to 50 age group (Kelsey, 1978). Despite efforts since the 1920's to curb the industrial back problem, its impact has continued to grow.

Completion of the study will yield sufficient information to allow us 1) to define the role of ultrasonic imaging of the lumbar canal in predicting back injuries; 2) to determine the relative importance of fitness, height, weight, sex, age, medical history, psychological stress, psychological traits, and social background in the development of back injuries and chronic disability; and 3) to evaluate the effect of loads on the lumbar spine imposed at the work site on the development of back injuries and chronic disability.

In the United States, the industrial back problem affects at least 2 percent of employees and their families each year, increasing the cost of industrial products worldwide. The knowledge generated from the present study will lead to the development and employment of logical strategies to lessen the burden of this significant health problem.

Publications - Abstract(s) available

Hansson TH, Bigos SJ, Wortley MD, Spengler DM: The load on the Lumbar Spine During Isometric Strength Testing. Spine, October 1984.

Batti'e MC, Hansson TH, Engel J, Zeh J, Bigos SJ: The Reliability of Measurements of the Lumbar Spine Using Ultrasound B-Scan. Spine, March 1986.

LIFTING CAPACITY OF INDUSTRIAL AND STUDENT POPULATIONS

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Musculoskeletal Injuries
1 R01 OH01956-01
09/01/84 - 12/31/85
$44,558 ($44,558 Cum)

Objectives

The major objectives of this research were to determine the difference between lifting capabilities (maximum acceptable weight of lift) of industrial and student populations, by collecting experimental data under identical conditions, and to quantify patterns of these differences.

Methodology

Using the psychophysical approach, 37 male and 37 female college students determined (subjectively) the maximum amount of weight they were willing to lift for a given combination of task variables. Three task variables will be studied: box size, lifting frequency, and height of lift. Their levels were identical to those used for industrial subjects in a previous study (NIOSH Grant No. 1 R01 OH01429-02). The experimental protocol used for student subjects was also identical to that used for industrial subjects.

As in the case of the industrial population, five male and five female students (a subject of the sample population) participated in the study to verify the psychophysical approach in manual lifting. The changes in their oxygen uptake and heart rate with the lifting duration were also determined.

Progress & Accomplishments

Project completed. Final report mailed 1/21/86.

Significance

The results of the study indicated that: The psychophysical approach is also not validated in case of student subjects. The decline of responses with time, however, is slower compared to industrial workers. This indicates that industrial workers overestimate their capacities more (compared to inexperienced students) when the psychophysical methodology is used.

Overall, male students lifted 89% of the weight industrial males lifted for 8-hours. For 12-hour duration, male students lifted 98% of the weight industrial males lifted. The corresponding numbers for female were 94% and 98%.

Generally, the student population response to task variables was similar to that of the industrial population.

In addition to the findings: Relationships between the two, populations, for various combinations of task variables, are provided.

Data bases for lifting asymmetrical loads are provided for industrial male and female workers.

Publications - None to date

BACK INJURIES: MECHANICAL STRESSES IN THE HUMAN SPINE

Albert Schultz, Ph.D.	Musculoskeletal Injuries
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University of Michigan	09/01/83 - 08/31/86
Ann Arbor, Michigan 48109-2125	$ 138,739 ($1,032,463 Cum)

*Previously supported under Grant Number R01 OH00514.

Objectives

The objectives of this research are: (1) to analyze the three dimensional force systems that act on the human spine during industrial handling and lifting tasks, (2) to analyze the mechanical stresses imposed on the structures of the trunk by those force systems, and (3) to apply this information to reduce industrial injuries.

Methodology

Basic features of the program are: (1) the use of computer-generated analogs of the entire thoracolumbar spine and of single substructures of the trunk to make the three dimensional force analyses, and (2) the conduct of experiments to provide input data for the models, to determine when the models are valid, and to confirm predictions made from model-obtained results.

Progress & Accomplishments

The progress and accomplishments are documented by the Publications listed below.

Significance

A large proportion of industrial injuries result from manual materials handling and lifting tasks. Back injuries occur with great frequency, often cause acute suffering and serious disability in the workers involved, and are extremely costly to the industrial economy. The results of these biomechanical studies should lead to significant improvements in the ability to reduce the incidence and severity of industrial back injuries.

Publications

Reuber M, Schultz A, Denis F, Spencer D: Bulging of lumbar intervertebral disks. J Biomech Engng 104:187-192, 1982.

Schultz A, Andersson G, Ortengren R, Bjork R, Nordin M: Analysis and quantitative myoelectric measurements of loads on the lumbar spine when holding weights in standing postures. Spine 7:390-397, 1982.

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Schultz A: Mechanical factors in the Etiology of idiopathic low back disorders. Symposium on Idiopathic Low Back Pain, American Academy of Orthopaedic Surgeons, St. Louis, 1982.

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IMPACT OF AUTOMATION ON INDUSTRIAL INSPECTOR STRESS

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Musculoskeletal Injuries
5 R01 OH02024-02
09/28/84 - 08/31/87
$87,579 ($179,300 Cum)

Objectives

The objective of this research is to determine how both human stress and human performance vary with the level of automation in an industrial inspection job. Industry is demanding higher quality of its workers and of its inspectors who are charged with maintaining and monitoring that quality. At the same time, sophisticated automation is becoming available to extend the human inspector's capabilities on some tasks within the inspection job. Inspection is known to be a stressful job and, with these two trends, a job which is likely to increase in stressfulness.

Methodology

An industrial inspection device is to be used to provide varying levels of automation. The Color Video Comparator (CVC) superimposes television images of the inspected item and a standard item in rapid succession onto a single color monitor. Any defects appear as changes in visual pattern between the successive images. In cooperation with AT&T Technologies, Inc., a stimulus set of life-sized photographs of a printed circuit board has been obtained. These photographs are of either a perfect board or one containing up to two of four different defects. Unemployed industrial workers are trained on this task, both using unaided visual inspection (the control condition) and at one of six levels of automation on the CVC:

1. Freely moving CVC to search board.
2. CVC moves in steps across board.
3. CVC moves in pre-determined step sequence.
4. Paced predetermined sequence, equal times for all areas.
5. Pattern recognition algorithm to automate search.

Performance measures are search time, stopping time and error rate. Stress measures are visual stress (Critical Flicker Fusion Frequency), postural stress (self-report scales and measures of ancilliary behaviors) and information processing load (heart rate variability, GSR). In the final year, AT&T Technologies is cooperating in replicating parts of the experiment using actual circuit board inspectors to give measures of job stress as well as task stress.

Progress & Accomplishments

The printed circuit boards, CVC and subject pool were obtained in Year 1 and the experiment at the first level of automation was run and analyzed. It was found that automation level 1 was slower than free inspection for stopping time ($p < 0.01$), but slightly better for defect detection ($0.05 < p < 0.07$ for three defects). Stress measures were not significantly different for this level of automation.

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As a result of this experiment and anlysis, the data collection has been expanded (GSR, Blood pressure) and shortened (2 vs 3 days). All levels of automation are being run again in year 2 with the new measures for consistency. Liaison is being maintained with AT&T Technologies for the final in-plant studies.

Significance

The results of the project will be of value to designers of automated and semi-automated systems as they will help to specify the level of automation which gives the best balance system performance and operator stress.

Publications

Drury CG, Kleiner BM: A comparison of blink aided and manual inspection using laboratory and plant subjects, Proceedings of 29th Human Factors Society Annual Meeting, San Antonio, pp. 670-674, 1984.

BACK PAIN IN INDUSTRY: A PROSPECTIVE EPIDEMIOLOGIC STUDY

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Musculoskeletal Injuries
1 R01 OH02084-01
12/01/84 - 11/30/87
$146,794 ($146,794 Cum)

Objectives

A prospective epidemiologic study of back pain in the industrial population of the Allen-Bradley is underway. The large scale study seeks to address deficiencies in characterization of indicants of individual and occupational "predisposition" to low back pain and to define rates of occurrence of low back pain. Specific variables to be assessed as potential risk factors include attributes of the individual, characteristics of the work environment and characteristics of occupational tasks and recreational activities. These variables are to be correlated with "episodes" of low back pain (resolution without specific treatment), "cases" of low back pain for which treatment is sought and the frequency and duration of work loss. Remedies sought by the employee are to be catalogued and the major descriptive outcomes of the study will include period prevalence rates, annual incidence rates and recurrence rates for the total population of employees and for various demographic subgroups in different job categories. Identification of major risk factors may enhance the likelihood of successful implementation of appropriate preventive strategies.

Methodology

Employees at the Allen-Bradley Company in the first year of the study complete a questionnaire administered by nurse-coordinators at the worksite. The questionnaire characterizes individual demographic characteristics, off work activities, pertinent medical history relating to low back pain and the employee's perception of job tasks. Anthropometric measurements are obtained on employees. Rates of occurrence of "episodes" of low back pain are to be determined by follow-up annual questionnaires and "cases" are to be identified at the time of presentation to the Medical Department of the Allen-Bradley Company. Specific characterization of occupational environment and tasks is to be accomplished during the second and third years of the study. Baseline and follow-up data are to be analyzed; both cross sectional and longitudinal associations are to be assessed. Analysis is to include a descriptive format, univariate and multivariate statistical techniques.

Progress & Accomplishments

In the first four months of data collection over 1,600 of the 4,000 employees in the study population have completed entrance questionnaires and undergone anthropometric measurements. Preliminary statistical evaluation is underway.

Significance

Back injuries are among the most frequent of work-related diseases and injuries, and spinal pain is among the most common of human afflictions. Costs are reflected in both human suffering and immense economic losses.

Epidemiologic data on low back pain have not been extensively developed in the United States; it is hoped that through identification of risk factors prevention of back pain and prevention of the progression of back pain to disability may become more likely.

Publications - None to date

ISOMETRIC VS. ISOKINETIC BACK LIFTING PERFORMANCE

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Musculoskeletal Injuries
5 R03 OH01755-02
07/01/83 - 10/31/85
$22,630 ($41,700 Cum)

Objectives

Existing knowledge of the biomechanical capabilities of the back during lifting activities is limited to static evaluations of exertions. This has resulted in ergonomic recommendations, models, and evaluative procedures which have produced little impact in the control of the low back pain (LBP) problem. The problem arose from the fact that manual material handling tasks usually require dynamic action of the biomechanical system. The objective of this research is to investigate biomechanical capability of the back in a quasi-dynamic testing procedure. This effort has facilitated the assessment of trunk loading costs when isokinetic trunk motion is included in the evaluation.

Methodology

A laboratory investigation which permits a biomechanical interpretation of the consequences of motion in the sagittal plane of the body was conducted. Forty-five male volunteers ages 17 to 61 served as subjects. In this experiment the trunk motion was controlled using an isokinetic dynamometer. This device permitted the monitoring of both static and isokinetic trunk exertions while the subjects were in back lift positions. Static exertions at 0, 22.5 and 45 deg. of forward trunk bend and isokinetic exertions of 15, 30 and 90 deg/sec of trunk motion through the 60 to 0 deg angle range were monitored. Trunk torque and back muscle electromyography (EMG) were recorded as the subjects performed the exertion.

Progress & Accomplishments

This project has been completed. The results reveal that significantly greater loadings occur under slow dynamic conditions compared with static conditions, indicating that static models are not good indicators of dynamic trunk load. The correlation between trunk torque and EMG in the erector spinae muscles also increased significantly as the trunk velocity increased indicating that EMG may serve as a good indicator of trunk exertions under dynamic conditions.

The relative trunk loading needed to support a constant torque under the various velocity and angle conditions were also investigated and described.

Finally, the time sequence of muscle loading activity was investigated. The peak trunk loading was similar under static and 30 deg/sec exertions but varied markedly under other conditions.

Significance

These analyses have demonstrated that the trunk loadings which occur under static conditions change markedly as velocity is introduced into a lifting analysis. Thus, lifting models which are based solely upon static exertions may misinterpret the true loading which occurs at the trunk during a lift. This research has demonstrated how such models may be adjusted to reflect the spine loading effects of motion during a sagittally symmetric task.

Publications - None to date

VIBRATION OF THE INTERVERTEBRAL MOTION SEGMENT

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Musculoskeletal Injuries
5 R03 OH01758-02
07/01/83 - 06/30/85
$21,988 ($39,534 Cum)

Objectives

The objective of this study was correlate mechanical changes occuring in the intervertebral motion segment due to exposure to seated-posture loading environments (e.g. seated factory or office work, or car and truck drivers) with epidemiologic data showing an increased incidence of herniated nucleus pulposus in seated humans in static or vibration environments. The results of this study help elucidate the mechanical factors involved in the production of low back pain in humans working in a seated environment.

Methodology

A systematic study has been conducted to assess the effects of the new parameters of loading rate and load exposure history upon the mechanical response of forty lumbar motion segments free to move in six degrees of freedom. Changes in mechanical properties (main and coupled compliance characteristics and the segment's balance point location) were assessed before and after cyclic or static load exposures via translation-load and rotation-load tests at three different loading rates. The concept of determining the mechanical properties of the motion segments by axial compressive loads applied at the mechanical balance point was evaluated and proved. The balance point chosen was that which minimized flexion-extension and lateral bend rotations in response to an axial compressive load applied to the motion segment. Motion segments demonstrated sensitivity to loads applied away from the balance point. Balance points were generally located behind the geometric center of the disc and changed their location with load exposure history. The results suggest the existence of other balance points associated with other applied loads or torques. The balance point concept has implications in the way in which motion segments are tested and the way in which the lumbar musculature supports the lumbar spine.

Progress & Accomplishments

Load exposure history consisted of loading the lumbar motion segments as they would be while sitting for one hour in either a static environment (office or factory seat) or a conservative vibration environment (car or truck driving). One-hour load exposures produced significant changes in the mechanical characteristics of the segments. After one-hour load exposure, the segments were subjected to a rapid, flexion-compression overload event to simulate a person catching a falling load. Further mechanical property changes were observed. Motion segments tested at various load rates exhibited coupled viscoelastic mechanical responses. During testing, some specimens exhibited a sudden large rotation in both flexion and lateral bend (up to physiologic limits). This response occurred more often in the cyclically loaded L2-3 motion segments. The postero-lateral region of the disc may be at risk during such sudden combined rotations. Segments tested have shown sensitivity to load rate, load vector (magnitude and orientation), and load history. This work has provided several unique and valuable contributions. First, it has tested and proved the existence and the value of a mechanical balance point in the in vitro lumbar motion segment. 2) A systematic study was performed of the effect of the new parameters of a) load rate and b) load history upon the six degree-of-freedom mechanical response of the L2-3 and L4-5 motion segments. 3) The load history used revealed that a one-hour duration exposure to the conservative combined compression-flexion loading found in both static and vibration seating environments caused statistically significant changes in the mechanical properties of the motion segment. It exhibited further changes occurring due to an overload of the segment following the one-hour seating exposure. 5) Data were obtained of the mechanical response of the motion segment, which will be useful to spine researchers and mechanical modelers. 6) The balance point is located posterior to both the geometric centers of the upper vertebral body and the point of intersection of the mid-transverse place of the disc and the line segment connecting the upper and lower vertebral body centers. It was located near the instant center found by Gertzbein, et al., (1984) using a more sensitive technique analyzing flexion-extension. 7) The balance point changes location in response to load history. 8) The segment exhibits coupled viscoelastic response characteristics.

Significance

This study has several implications. Mechanical properties of a intervertebral motion segment should be obtained at mechanically based references (such as the balance point) as opposed to geometrically based ones. This has the possible benefit (although not yet tested) of simplifying the existent stiffness matrices used to describe the mechanical properties of the motion segment. It may also help reduce some of the scatter in mechanical properties reported by Tencer and Ahmed (1981), Schultz, et al., (1979) and Berkson, Nachemson and Schultz (1979). Testing motion segment properties at a balance point would also provide a reference for obtaining data comparable between researchers.

There are also several clinical implications of the work. 1) Exposure to seating environments causes statistically significant changes in the mechanical properties of the motion segment. This may indicate seat and job task redesign, but more work is needed to evaluate the long-term mechanical changes. 2) Neuromuscular control systems may use a balance point for applying loads properly to the spine. Due to the mechanical sensitivity of a balance point, it is important that the muscles apply the loads properly. A small mismatch (due to chronic or acute, trauma or disease processes) between the way the load is applied and the way it "should be applied", as dictated by the segment's properties, may lead to a condition where the muscles may enhance damage of the segment. 3) The segment is sensitive in several directions to load application rate. This suggests that low back pain sufferers should not rapidly change complex postures. For instance, one should get in and out of a car seat slowly, using simple posture changes. 4) It may not be wise to rapidly lift and twist after driving as the rotational compliance of the L4-5 motion segment increases (gets softer) due to vibration.

Publications - Abstract(s) available

Wilder, DG: On Loading of the Human Lumbar Intervertebral Motion Segment. Dissertation for degree of Doctor of Philosophy, Civil and Mechanical Engineering Department, University of Vermont, October 1985. Manuscript in press, University Microfilms International, Ann Arbor, 1986.

CARPAL TUNNEL SYNDROME - INTRINSIC RISK FACTORS

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Objectives

The evaluation of Carpal Tunnel Syndrome (CTS) in the workplace has focused mainly on repetitive movements of the wrist, performance of tasks with the wrist in ulnar deviation and chronic exposure to vibration. In addition to the examination of these extrinsic factors, association of CTS with intrinsic conditions, such as arthritis, diabetes, wrist trauma, hypothyroidism and hormonal status of women have been described. The purpose of this study is to examine any relationship that carpal canal size and the development of CTS may have on workers in one manufacturing environment. Specific objectives include:

1. To examine the association of cross-sectional area of the carpal canal as determined by the digitization of computerized axial tomography of the wrist with the presence or absence of CTS in an entire work population.
2. To determine if there are other critical measurements of the carpal canal which correlate with the presence of CTS, namely, the depth, width, and shape (longitudinally and cross-sectionally) of the canal.
3. To determine a prevalence rate for CTS in the population of workers in this study.
4. To explore if there is a carpal canal measurement or size which significantly increases the risk of CTS in workers who perform specific hand movements.

Methodology

The study design incorporates an historical prospective format in which an entire group of employees in one industrial setting, classified in the same job category, performing identical jobs with similar hand tools were examined. All recruited individuals were given a symptom questionnaire and clinical history, standard physical examination, electro-diagnostic studies and computerized tomography of both wrists. Any worker identified as positive for two of the three diagnostic tests (by specific criteria) were defined as CTS cases.

Computerized tomography measurements were performed on each individual prior to the identification of CTS. Six measurements were made on each wrist in a standardized manner for circumference, depth, width, and shape (longitudinally and cross-sectionally). Preliminary analysis evaluated individual measurements for cases compared to controls. Linear regression will explore any differences in dimensions of cases and controls. A prevalence rate of CTS will be determined for this manufacturing facility.

Progress & Accomplishments

To date, a total of 92 employees engaged in identical work in one manufacturing company have participated in the health screening for CTS. Initial screening and recruitment were completed in the Employee Health Unit and with subsequent diagnostic studies at the Baltimore City Hospital's

Neurology Laboratory. Delays resulting from lay-offs and sick leave forced extensions for both phases of employee participation. No additional participants will be recruited or completed at this time.

Results of the medical evaluations have been interpreted and coded according to the established procedures for this project. The interpretations of the computerized tomography were delayed, however, all quantitative measurements for depth, circumference, and width on each of six (6) slices for each wrist of each participant have been completed. All data have been coded, entered, and verified. Statistical and epidemiological analyses of demographic characteristics of this study population have been completed. Additional analyses of significant results are still continuing as well as programming for several unique analyses.

Significance

This study will explore an important intrinsic risk factor not previously examined in the workplace. The carpal canal can now be measured using computerized axial tomography. In-depth examination of various cross-sectional measurements of the carpal canal in both men and women may be beneficial in understanding the development of CTS in jobs that require repetitive hand and wrist movements. Secondly, this study will identify a prevalence rate in a group of male and female workers who perform identical jobs with their hands and wrists by diagnostic parameters.

Publications

Bleeker ML, Bowman M, Moreland RF, Tipton A: Carpal Tunnel Syndrome: Role of Carpal Canal Size. Neurology 35:1599-1604, 1985. (Partial Support)

QUANTIFICATION OF DYNAMIC MUSCLE STRENGTH

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Musculoskeletal Injuries
5 R03 OH01802-02
07/01/83 - 08/30/85
$12,650 ($27,370 Cum)

Objectives

The objectives of the study were: 1) to compare dynamic strength data obtained from a Cybex isokinetic dynamometer and a Mini-Gym and 2) to devise a set of dynamic strength tests appropriate for industrial use and to compare these to existing static and psychophysical tests. The first device is very accurate and adjustable but is also very expensive and cumbersome to carry into the field. The second device offers portability and simplicity.

Methodology

A two phase study was carried out. In Phase I, flexion and extension strengths of the elbow, shoulder, truck, hip, and knee were measured on five males and four females on both the Cybex and the Mini-Gym. Joint torques and joint positions were measured continuously over time over a variety of different angular velocities. In Phase II, dynamic lift tests of the leg, torso, and arm were devised. Ten males and ten females performed three repetitions of the three dynamic tests as well as three repetitions of the three comparable static and psychophyscial tests.

Progress & Accomplishments

In Phase I, typical torque over range of motion data indicated an inverted-U shaped curve with torques doubling from a low value at one extreme angle to the high point at mid range of motion, decreasing thereafter. Peak torques decreased with increasing angular velocities. There were large significant differences in the isokinetic data as collected on both devices. Only certain types of movements could be measured on the Mini-Gym. Peak forces were typically lower on the Mini-Gym. The lack of a fixed moment arm on the Mini-Gym allowed other muscle groups to contaminate specific joint movements and degraded the desired angular motion. In Phase II, there was a significant difference in strengths by the type of test and the body part tested. Dynamic strength was on the average 88% of static strength and more than twice as large as psychophysical strength. Variability in dynamic tests was not significantly different from the other two types of tests.

Significance

Accepting its limitations to use on only certain types of gross body movements, the Mini-Gym still has certain advantages. It is inexpensive, simple and portable. Several repetitions of tests can be run very quickly and it is completely under voluntary control. Combined with the three dynamic lifting tests, the Mini-Gym dynamic strength tester can be an acceptable device for measuring strength in the industrial environment.

Publications

Menon K, Freivalds A: Repeatability of Dynamic Strength Tests. Proceedings of the Human Factors Society (29th Annual Meeting), p 517-520, 1985.

REFLEXIVE AND TACTILE EFFECTS OF HAND TOOLS

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Musculoskeletal Injuries
5 R03 OH01852-02
05/01/84 - 04/30/86
$6,000 ($23,536 Cum)

Objectives

The purpose of this work is to investigate how tool handle design affects stresses in workers' hands. It is hypothesized that power hand tool vibration influences grip exertions, affects forearm muscular activity, impairs hand tactility, causes discomfort and localized muscle fatigue for inexperienced operators.

Experiments were performed to accomplish the following objectives:

1. Determine the effect of hand tool vibration (acceleration, frequency and direction) and tool weight on hand grip compression force under typical hand tool muscle loading conditions.
2. Determine the effect of vibration on muscular contraction of extrinsic hand flexor and extensors in the forearm while controlling hand grip compression force.
3. Determine the effect of vibration exposure on tactile sensitivity, subjective measures of discomfort and onset of localized muscle fatigue.

Methodology

A survey of industrial power hand tools was taken including a variety of hand held rotary and reciprocating tools. The conditions of laboratory experiments corresponded to the vibration features of many of these tools.

A hand tool simulator was constructed to produce vibration over a range of amplitudes and frequencies, in any one of three orthgonal directions. A servo controlled counterbalance system supported the entire apparatus to control its weight and loading on the hand and arm. The simulator handle was instrumented with a strain gage dynomometer to measure hand grip compression force.

Surface electrodes were used to record electromyograms from forearm muscles while subjects grasped the tool simulator handle at required hand grip exertions using visual feedback.

Subjects operated the simulated power tool for a period of time then measuring tactile sensitivity and indices of discomfort and localized muscle fatigue. Tactility was measured recording ascending and descending thresholds of ridge detection and two point discrimination. Onset of muscle fatigue was determined using electromyogram rms amplitude, mean power frequency, and muscle tremor. Discomfort was assessed using subjective responses in a cross modality matching task.

Progress & Accomplishments

Laboratory and field work has been completed and data analysis is in progress.

Significance

Cumulative trauma disorders are a major health problem among workers in manual industries. The application of excessive hand grip force can contribute to the incidence of cumulative trauma disorders such as carpal tunnel syndrome, cause discomfort and localized muscle fatigue, and impair workers' ability to perform delicate assembly operations.

Publications - None to date

REPETITIVE TRAUMA DISORDERS: OCCUPATIONAL RISK FACTORS

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Musculoskeletal Injuries
1 R03 OH01906-01
09/30/83 - 03/31/85
$15,828 ($15,828 Cum)

Objectives

1. To determine the prevalence of cumulative trauma disorders of the hands, wrists, forearms and elbows among workers in a plant manufacturing plastic automobile components.

2. To estimate the relative risk of selected disorders among workers performing jobs with defined physical attributes including forcefulness, repetitiveness and postural requirements.

Methodology

A brief medical history questionnaire and limited physical examination were used to determine the prevalance of selected hand, wrist, forearm and elbow problems among workers in different departments and major job groups. A group of cases was selected along with controls having no upper extremity complaints. Recent jobs performed by cases and controls were observed and analyzed for characteristics believed related to cumulative trauma disorders. Job analysis was done utilizing a simplified observation checklist derived from more elaborate biomechanical evaluations in use by other researchers. Exposure variables derived from this job analysis will be used for hypothesis testing.

Progress & Accomplishments

The prevalence evaluation has been completed, cases and controls have been identified, and jobs have been analyzed for ergonomic risk factors. Prevalence evaluation revealed that 52% of 574 workers surveyed had at least one of seven specific cumulative trauma disorders, the most common being epicondylitis (16%) and hand/wrist tendinitis (19%). Although no work area in the plant had a prevalence of less than 30% among either men or women, the disorders were not evenly distributed. Sex specific and sex adjusted prevalence odds ratios were used to estimate the risks of cumulative trauma disorders in specific departmental groups relative to all other departments in the plant combined. The risk to workers for having any of the disorders was greatest in Foam Assembly where the sex adjusted odds ratio was 2.77 ($p < 0.05$). Specific significant associations included nerve entrapment at the elbow among both sexes in Foam Assembly and hand/wrist tendinitis among men in Cluster Assembly. The impact of these disorders was estimated by an evaluation of worker-reported lost work days, job changes and medical treatments. Final data files are being completed prior to case control analysis.

Significance

If exposure variables are, as hypothesized, associated with cumulative trauma disorders, it will be possible to incorporate simplified methods of job analysis into prospective ergonomic intervention programs in which job risk scores are used to identify activities most likely to cause health problems and to thereby allow plant health and safety personnel to target high-risk jobs for engineering change.

Publications - None to date

AFLATOXIN EXPOSURES OF AGRICULTURAL WORKERS II

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Occupational Cancers
5 R01 OH00796-03
09/01/82 - 02/28/85
$78,862 ($78,862 Cum)

Objectives

1. To develop an analytical method capable of detecting aflatoxins in dust collected at the breathing zone of workers by personal sample devices.
2. To conduct a field survey to determine inhalation exposures of workers to aflatoxins in contaminated corn.
3. To determine the concentration of viable aspergillus fungi in contaminated corn.
4. To determine the size distribution of the aflatoxin-containing dust from corn handling.
5. To conduct a feasibility study to determine whether a suitable population existed for an epidemiologic study of corn handlers.

Methodology

The project was done in cooperation with the Department of Agriculture's Regional Research Laboratory in Peoria, Illinois. Samples collected in the corn harvest in Georgia were sent to the Peoria laboratory. A scientist was assigned to the Peoria lab to develop a more sensitive analytical method under the direction of Dr. Odette Shotwell.

"Field" samples in this study were collected in corn fields (on the combine) and at the corn elevators during unloading of corn. Personal samples were taken from sampling devices on the shoulders of workers, area samples were taken at various locations on the combine and in the elevator. High volume cascade impactor samples were taken to determine the size distribution of the dust, and of the aflatoxin-containing dust.

For more detailed characterization of dust from corn handling, bulk samples were brought from Georgia to Cincinnati for laboratory simulation of corn handling. Samples were loaded in a modified tumbling mixer from which all dust emissions could be collected. The dust emitted during successive time periods was measured. These dust samples were also analyzed for aspergillus spore emission. Size distributions were done on dust generated during the handling, using the same high volume cascade impactor which had previously been used in the field sampling.

A list of corn handling facilities was obtained from the Georgia Department of Agriculture, and these facilities were contacted by telephone to ascertain employment, length of records, and whether they would be willing to participate in a worker health study by furnishing employment records.

Progress & Accomplishments

Previous years of the grant had found (a) an analytical method that could be used on personal dust samples from workers, (b) that there were indeed measurable concentrations of aflatoxin in corn dust generated during handling, (c) that the dust was predominantly in the "coarse" fraction, i.e., that which is primarily filtered out in the nasopharyngeal regions, and (d) that aflatoxin concentrations in corn dust were extremely variable.

The laboratory corn handling project was completed. It was found that, as in field samples, most of the dust (> 90%) was in the "coarse" fraction. The aflatoxin concentration in coarse and fine dust was comparable. Dust emission from the corn during handling was found to decrease approximately as the square root of time, e.g., after four minutes of handling the dust concentration was about half that after one minute of handling.

It was found that 60 percent of the surveyed Georgia grain handling facilities had 10 or fewer employees, and 83 percent had 20 or fewer. Of the respondents, 55 percent had records for 10 years or less, and 25% had records of 25 or more years or employment. It was concluded that an epidemiologic study, if conducted, should not depend upon grain handling facility records.

The nature of the dust and its contaminant concentrations lend themselves to control by simple respiratory protection. If, when a farmer or corn-handling employee is in a highly visible cloud of dust, he/she wore a well-fitting NIOSH/MSHA respirator approved for pneumoconiosis-producing dust, calculated exposures would be no higher than those of peanut-butter eaters.

Significance

This project has demonstrated that significant aflatoxin exposure from dust generated during handling of aflatoxin-contaminated corn is possible. It has also shown that such dust is relatively coarse, so that any potentially hazardous dust cloud would be readily visible. Protection from any potential hazard could be obtained by wearing a well-fitting, simple dust respirator when in visible dust. Because the time in dust would be limited, the respirator-wearing periods would be within those which people have been shown to tolerate. The pilot feasibility study suggested that it will probably never be possible to prove (or disprove) a lung and/or liver cancer hazard from handling aflatoxin contaminated corn. The prudent farmer or grain-handling facility operators will take steps to protect themselves and their employees from the aflatoxin and any hazard which it may present.

Publications

Creagh EM: A Characterization of Aflatoxin Emissions from Contaminated Corn. Thesis MS, University of Cincinnati College of Medicine, 1985.

CARCINOGENIC PROPERTIES OF NITROUS OXIDE

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Occupational Cancers
5 R01 OH01177-03
08/01/82 - 07/31/85
$ 43,871 ($114,593 Cum)

Objectives

The primary aim of this project is to perform in vivo studies to determine whether nitrous oxide, the most commonly used inhaled anesthetic in the United States, is carcinogenic to experimental animals. A secondary aim is to use the results obtained to predict whether there are feasible methods for reducing the full histopathological examination in carcinogen bioassay.

Methodology

Groups of 75 male and 75 female Swiss/Webster were exposed to either 10 or 40% nitrous oxide or to air. Exposure was 4 hours per day, 5 days per week. In a previous subchronic study, 40% nitrous oxide was determined to be the maximum tolerated does for this strain of mouse. After 78 weeks of exposure, a 5-week period without treatment was allowed to insure that examination for carcinogenicity was not confounded by acute toxic changes. All animals killed at the scheduled time or dying in extremis at other times were subjected to complete autopsy examinations. The only exceptions were eight mice in which cannibalism or advance autolysis precluded examination. The procedures used for the gross autopsy were the same as those recommended by the National Cancer Institute and have previously been described in detail. More than 40 tissues were examined in situ, then dissected form the carcass, incised, and reexamined before being fixed 10% neutral buffered formalin. Liver, spleen, kidneys, and tests were weighted fresh. After fixation, tissues were again examined grossly, and 32 routine sections from different organs and all abnormal tissues were processed for microscopic examination. Included in this group were bone marrow and blood smear (the latter for red cell, differential white cell, reticulocyte, and platelet counts). Tissue sections were cut at 4 to 6 um and stained with hematoxylin-eosin. Microscopic examinations were performed by a pathologist experienced in mouse histology. Examiners were unaware of the treatment groups of the mice at the time of all examinations. Consultation was sought from other pathologists when a histopathologic diagnosis was in doubt. Intergroup comparisons were made using analysis of variance with post-hoc tests, probit analysis and Chi-square test as appropriate; $P < 0.05$ was considered statistically significant.

Progress & Accomplishments

The primary aim of determining the carcinogenic potential of nitrous oxide has now been accomplished. In brief, results of the study were as follows. Mean body weights for males and females in the 10% nitrous oxide group were similar to those in the control group throughout the study, whereas they were about 5% less for the 40% nitrous oxide after about week 60 of gassing for males and week 40 for females. No other gross toxic effects of treatment were noted. Mean organ weights of nitrous oxide treated mice were not statistically different from those of control mice nor were mean organ weights expressed as a percentage of body weight different from controls. The survival rates for all groups throughout the study were the same after an adjustment was made for a small number of animals that died of hyperpyrexia during one gassing run. Gross and microscopic examination of tissues revealed a variety of non-neoplastic lesions including ovarian cysts, cholecystitis, bladder stones, and testicular atrophy. Their presence was unrelated to treatment. In general, there was no microscopic evidence of cellular damage and, in particular a detailed examination of blood smears and bone marrow section showed no evidence of megaloblastic changes or bone marrow depression. The first neoplastic lesion appeared 26 weeks after the start of treatment, and numerous other tumors appeared throughout the study. As in similar studies with other anesthetics, most of the tumors in both control and test animals were either lung adenomas of alveolar cell origin or liver tumors of the basophilic hepatocellular adenoma type. There were no statistical differences among the groups either in the total number of tumors or in the number of tumors of a particular cell type. It should be noted, however, that the background incidence of lung adenomas in this study was high and thus the statistical power for the analysis of differences among the groups for this tumor type was low.

Significance

Nitrous Oxide is the most commonly used inhaled anesthetic in the United States. Furthermore, it is the most abundant nitrogen contaminant of our atmosphere and is even present in low concentrations in clean air because of production in the soil. It is therefore encouraging for operating room personnel, surgical patients and even the general public that our study is negative. In conjunction with results from other studies, the results from our study in which nitrous oxide was administered to mice for 18 months at the maximum tolerated dose suggests that it has no carcinogenic potential and no significant non-hemopoietic chronic toxicity.

Publications - Abstract(s) available

A CARCINOGEN/MUTAGEN DETECTOR FOR HPLC

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Occupational Cancers
5 R01 OH01331-03
08/01/82 - 08/31/85
$109,050 ($321,625 Cum)

Objectives

The objectives of the Carcinogen/Mutagen Detector project were:

1. develop a pulse-conductivity detection system that is selectively sensititive to carcinogens/mutagens (C/M's) and is compatible with HPLC;
2. test the sensitivity and specificity of this system to biohazards that commonly occur in the workplace;
3. apply this system to monitoring complex mixtures of workplace biohazards either prepared synthetically or sampled in the workplace and compare with Ames-test response;
4. determine if the correlation between the electron-attachment rate constant, k_e, and carcinogenicity also applies to k_e-teratogenicity; and
5. determine the theoretical basis for the k_e-C/M correlation.

Methodology

The C/M detector is based on using the electrophilic properties of a chemical as a measure of its carcinogenic potential, which is consistent with the tenet of J.E. and E.C. Miller that "an initiating chemical (carcinogen has the special property of being a strong electrophilic reactant and thus of being able to combine chemically with necleophilic sites in the target cells..." [Cancer 47, 1058 (1981)]. The technique that we employ to probe the electrophilicity of a chemical is pulse conductivity in which a pulse of ionizing radiation is used to generate quasifree electrons, and from the kinetics of decay of these electrons an electron attachment rate constant, k_e, is derived. The C/M detector can be viewed as a continuously operating pulse-conductivity system that monitors the electrophilicity of chemicals flowing through it. Further, by using the C/M detector in conjunction with HPLC, which is the methodology used in analytical chemistry to separate a mixture into its component chemicals, the electrophilicity of each component of a complex test mixture can be monitored (Objective 1).

Another methodology that we employ both to corroborate the response of the C/M detector (Objective 3) and to attempt to determine the theoretical basis of the k_e-C/M correlation (Objective 5) is the Ames Salmonella test. This bioassay is the short-term test that is most widely used to screen chemicals for carcinogenic potential and has been alluded to by B.A. Bridges as "a very sensitive way of detecting electrophilic reagents" [Nature 261, 199 (1976)]. Thus, by combining this biological measure of electrophilicity with the physico-chemical electrophilicity measured by k_e, we are using a two-pronged approach to elucidate the initiating step in chemical carcinogenesis (Objective 5).

Progress & Accomplishments

Upon initiating this project in August, 1982, the proposed C/M monitor was redesigned to enable us to strive toward the objectives while operating within the constraints imposed by funding of the project at 80 percent of the level requested. The major components of the C/M monitor, which are a flash x-ray unit, a one-curie strontium-90 beta radiation source, and an HPLC system, were acquired and thoroughly tested. An ion chamber compatible with the HPLC system and a mechanical chopper and enclosure for the strontium-90 source were fabricated and tested. The one-inch Lucite walls of the ^{90}Sr enclosure provided adequate shielding of project personnel from beta radiation; however, bremsstrahlung produced by beta irradiation of the aluminum components of the chopper required that additional shielding be used. The requisite lead shielding was obtained and the C/M monitor was prepared for the final stages of testing when funding of the project terminated in August, 1985.

Concurrently with the development of the C/M monitor, progress was made in more firmly establishing the k_e-carcinogenicity correlation and in using and obtaining a better understanding of the Ames test. Concerning the former, the k_e's of more than 170 chemicals which had been classified as carcinogens or noncarcinogens in animal-test studies were measured, and the results indicated that the predictivity of the k_e test in screening carcinogens was equal to or better than the Ames bioassay. Our Ames-test studies were focussed on the response of the Ames tester strains to the most ubiquitous and well characterized human carcinogen--ionizing radiation. Our progress in these areas is demonstrated in the attached listing of Publications which includes references to our k_e measurements and the C/M detector reported by R.M. Baum and D.J. Brusick.

Significance

The significance of the Carcinogen/Mutagen Detector project can be succinctly expressed by again quoting Bridges: "...even an empirical 'litmus paper test', with no known theoretical basis, which gave an 80 to 90% predictiveness for carcinogenicity would be a powerful tool in the screening of chemicals for human toxicity" (loc.cit). The k_e-carcinogenicity correlation, which is the basis of the C/M detector, yields "an 80 to 90% predictiveness for carcinogenicity" for the >170 chemicals tested and thereby fulfills Bridges criterion for predictivity and thus should be considered as a powerful tool that could be exploited to reduce human exposure to potentially carcinogenic chemicals.

In addition to this pragmatic value of the C/M monitor, we are striving via Objective 5 to elucidate the theoretical basis of our "litmus paper test", and reaching this goal would undoubtedly by the most significant aspect of our work.

Publications - Abstract(s) available

Isildar M, Bakale G: Radiation-Induced Mutagenicity and Lethality in Salmonella Typhimurium. Proceeding of the Seventh International Congress of Radiation Research. JJ Broerse, GW Barendson, HB Kal, AJ van der Kogel, Eds., p. B4-19 M Nijhoff, Amsterdam, 1983.

Isildar M, Bakale G: Radiation-Induced Mutagenicity and Lethality in Ames Tester Strains of Salmonella. Radiat Res 100:396-411, 1984.

Isildar M, Bakale G: Comparative Lethal Effects of UV and Ionizing Radiation in Ames Tester Strains of Salmonella. Radiat Res 103:461-465, 1985.

RISK OF PATHOLOGISTS EXPOSED TO FORMALDEHYDE

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Occupational Cancers
5 R01 OH01511-02
09/30/83 - 05/30/86
$144,728 ($267,067 Cum)

Objectives

Recent studies by the Chemical Industry Institute of Toxicology (CIIT) suggest that exposure to formaldehyde vapor may be carcinogenic in rats. These findings have raised concern for the health of 1.75 million workers with occupational exposures to formaldehyde as well as persons with exposure from home insulation.

Methodology

This proposal is to examine the mortality experience of pathologists, a medical specialty group with formaldehyde exposure, using a non-concurrent prospective study design. Comparison populations include other physician specialists who have only limited exposure to formaldehyde. All causes of death listed on death certificates will be examined, and selected causes, notably cancers, will be verified.

Progress & Accomplishments

The study population has been constructed from lists of members in pathology associations. The vital status of the exposed and control populations has been ascertained, and death certificates to validate vital status and cause of death have been obtained. Data are being coded for computer entry for analysis.

Significance

Results from this study will help establish specific risks, if any, and levels of risk for humans with formaldehyde exposure.

Publications - None to date

OCCUPATIONAL EXPOSURE IN ADULT GLIOMA PATIENTS

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Occupational Cancers
5 R01 OH01557-04
09/29/82 - 08/31/86
$72,582 ($318,304 Cum)

Objectives

A large amount of suggestive evidence on the etiology of brain tumors has come from recent studies of the rubber, plastics and petrochemical industries. These studies suggest associations between chemical exposures and tumors of the glioma series. The objective of the present research is to pursue the findings of these occupational studies by conducting a case-control study of adult glioma patients. The proposed study will collect extensive occupational and residential histories for male and female glioma patients by interview and will compare the occupational distributions to matched neighborhood controls. Chemical exposures of interest will be followed up to identify possible agents.

Methodology

Eligible cases for the current study are adults with glial tumors of the central nervous system who are residents of Northern California or Northwestern Nevada at the time of diagnosis. An attempt to population-base a subset of cases in the study through the San Francisco-Oakland SMSA SEER Registry was judged not feasible because of the Registry's lag time in assembling incidence cases in a usable form and the limitation imposed by the mean survival time of 12 months for glioma patients. We proposed an alternative of searching the California State Mortality Tapes for brain tumor deaths, identifying adult gliama cases for the Bay Area counties, and locating a next-of-kin for proxy interview. One neighborhood control, matched to the case for sex, age, and race, is interviewed for each completed case interview. Occupational histories taken from completed interviews are coded by two measures of exposure. One measure is the industry code from the Standard Industrial Classification Manual. Each industry is coded independently by two coders and discrepancies are resolved by a metacoder. In the second method, two independent coders read job histories in questionnaire and assign 9-digit Dictionary of Occupational Titles (DOT) codes to each job. A reviewer reads DOT descriptions for all codes assigned and groups them into exposure categories. A judge blind to subject reported exposures and case-control status resolves exposure-category discrepancies.

Progress & Accomplishments

As of January 1, 1986, 512 cases have been entered into the study, of which 395 had been interviewed, 20 had consented and been assigned for interview, 50 were pending consent, 16 were ineligible or unlocatable since initial study entry, and 21 individuals had refused to participate. Proxy interviews were conducted for 162 of the 395 cases. There are 263 controls. Completion of coding for this data set is currently underway in preparation for analysis. A preliminary analysis has been performed on a subset of this data.

Preliminary results based on 124 pairs of case interviews and their matched control interviews show some differences between cases and controls in the categories of interest to the study hypothesis. Only two petrochemical workers appear in these 248 subjects but both are cases. When we group exposure categories to create a broader category of work with exposure to petroleum products combining the categories of petrochemical workers, mechanics, gas station employees, and professional drivers we find 26 cases and 15 controls with a history of one or more jobs in those categories. The one-sided p value for these numbers is 0.04. Exposure to cleaning solvents shows 8 cases and 4 controls. Metal fabrication, including welding, cutting, and assembly had 7 cases and 2 controls. Rubber working has one case and no controls. Job-related exposure to radiation shows 9 cases and 5 controls. Photographic chemicals, 3 cases and 1 control. No plastics or vinyl chloride workers have appeared yet in the study. There is no difference between cases and controls on exposure to paints and dyes, construction-related exposures, farm work, machine and lubrication oils, and the category of miscellaneous exposures. The category of no exposure was quite evenly distributed among cases and controls (46 cases and 52 controls).

Significance

The results outlined above are preliminary and the numbers are quite small (most of them are not statistically significant); nevertheless, some of them are suggestive and tending in the direction of the study hypothesis. Larger numbers will resolve some of the marginal findings in this ever versus never worked analysis and also permit us to look at duration of employment with a given exposure category.

Publications

Moss AR: Occupational exposure and brain tumors. J of Toxicology & Environ Health 16:703-711, 1985.

PRODUCTION AND FATE OF HYDROXAMIC ACIDS IN HEPATOCYTES

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Occupational Cancers
5 R01 OH02027-02
08/01/84 - 07/31/87
$62,438 ($128,180 Cum)

Objectives

The objective of this program is to determine the extent to which thiamine-dependent enzymes participate in the toxification or latentiation of C-nitroso aromatic chemicals in primary cultures of rat liver hepatocytes. Specific aims relevant to the elucidation of this objective are to 1) identify the hydroxamic acids produced from selected nitroso substrates by hepatocyte cultures, 2) determine the metabolic fate of such hydroxamic acids in hepatocytes and 3) determine the relative genotoxicity of the various types of hydroxamic acids of interest, particularly with respect to covalent binding to nucleic acids and protein.

Methodology

Rat hepatocytes in primary culture are prepared under aseptic conditions and used within four days following the initiation of such monolayer cultures. Chemical substrates containing the C-nitroso group on an aromatic ring are added to such cultures, and their metabolic fates are followed by use of high pressure liquid chromatography, and in certain cases by radiation dosimetry. Particular effort is made to determine the metabolic production of certain hydroxamic acids, which are indicative of the action of particular thiamine-dependent enzymes on nitroso substrates. Authentic chemical references of such metabolites of interest have been or will be prepared by common synthetic organic methods. These reference standards are employed to confirm the production of the metabolites of greatest interest. The biochemical fates of the hydroxamic acid metabolites are also examined by such techniques. Particular effort is made to determine the extent of metabolic activation of the hydroxamic acids, and subsequent covalent binding of them to cellular molecules such as DNA, RNA and protein.

Progress & Accomplishments

Studies with five selected C-nitroso aromatic compounds in rat hepatocyte suspension cultures demonstrated that the nitroso functional group is highly reactive in a metabolic sense. Within one hour all nitroso substrates had been consumed, with concommitant appearance of expected major metabolites such as the corresponding amines, acetanilides and arylhydroxylamines; however, the latter initial metabolites were short-lived in the conversion of arylhydroxylamines to other products was a relatively rapid reaction.

More detailed studies were conducted with plated hepatocytes (primary hepatocyte cultures) on 4-nitrosobiphenyl and particularly on 4-chloronitrosobenzene. With this more refined metabolic system, the nitroso functional group still remained quite reactive. Most importantly, the conversion of 4-chloronitrosobenzene to its N-glycolyl type of hydroxamic acid primary hepatocyte cultures was positively confirmed by use of a diode array rapid-scanning UV detector with high pressure liquid chromatography.

The percent conversion of this nitroso substrate to the glycolyl-hydroxamic acid was quite low, which was not surprising in view of the numerous pathways of metabolic conversion to which to nitroso functional group is susceptible. Furthermore it was possible to enhance the percent production of the glycolyl-hydroxamic acid through the use of certain modulators of metabolic reactions. Pentachlorophenol is an inhibitor of sulfation and was found to increase the production of the glycolyl-hydroxamic acid from 2-5 times depending upon the concentration of nitroso substrate that was employed. This suggests that the glycolyl-hydroxamic acid metabolite is further metabolized by O-sulfation. Similarly, depletion of glutathione (diethyl maleate, buthionine sulfoximine) and inhibition of glucuronidation (galactosamine) also resulted in increased amounts of the glycolyl-hydroxamic acid, although the enhancement was less than observed for pentachlorophenol.

A very notable observation was that the percent conversion of the nitroso substrate to the glycolyl-hydroxamic acid increased markedly rather than linearly as the concentration of the substrate was increased. Thus at an initial substrate concentration of 0.1 mM the percent conversion to the glycolyl-hydroxamic acid was 0.02 - 0.05%, while a concentration of 0.2 mM was converted to the this hydroxamic acid in a yield of about 0.2%. In the presence of 0.1 mM PCP the percent yield was further increased to 0.3%. The major fate of the 4-chloronitrosobenzene substrate in primary hepatocyte cultures was found to be loss by volatilization from the aqueous media. Thus, in a closed system, such as in vivo, it is to be expected that the percent conversions of this substrate to the glycolyl-hydroxamic acid would probably be several times higher.

Significance

This study has confirmed the original hypothesis that certain thiamine-dependent enzymes are involved in the production of structurally unique metabolites of nitroso compounds. In turn, the nitroso compounds are known intermediary metabolites of arylamines and related chemicals; therefore, it is expected that the unique hydroxamic acids derived from the action of transketolase on nitroso compounds will also be metabolites of the arylamine class of chemicals. Even though the amounts of such hydroxamic acids that are produced are very small, it is quite possible that these minor metabolites play an important role in the overall toxicological properties of many industrial and agricultural chemicals. Studies to elucidate the genotoxicity of such metabolites have not been completed.

Publications

Corbett MD, Corbett BR: Enzymatic N-oxidation of 4-nitroaniline. Biochem Arch, 1:115-120, 1985.

Corbett MD, Corbett BR: The reactions of C-nitroso aromatics with α-oxo acids in Biological Oxidation of Nitrogen in Organic Molecules (J.W. Gorrod and L.A. Damani, eds.), Ellis Horwood Ltd., Chichester, p 400-408, 1985.

OCCUPATIONAL CANCER SURVEILLANCE: NEW APPROACHES

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Occupational Cancers
5 R01 OH02067-02
09/28/84 - 08/31/87
$238,477 ($377,784 Cum)

Objectives

There are six specific scientific aims for this project:

1. A method of monitoring occupational cancer risks will be developed and assessed in terms of its feasibility for routine surveillance and in terms of its cost effectiveness.
2. The utility and feasibility of routine occupational surveillance among patients diagnosed with rare forms of malignancy, as well as with the more frequently occurring malignant tumors, will be assessed.
3. Occupational risks of blacks and females will be assessed, in addition to those of white males.
4. Brief smoking histories will be obtained as an essential complement to the occupational history.
5. Various statistical methods will be tested for their ability to produce results useful for generating hypotheses about potential occupational risks of cancer occurrence.
6. An ongoing, routine system of occupational monitoring will be intergrated with the Metropolitan Detroit Cancer Surveillance System (MDCSS), a participant in the NCI SEER program.

Methodology

This is a population-based study of all newly diagnosed cancer cases for 9 selected sites or types: bladder, colon, rectum, lung and bronchus, esophagus, liver, salivary gland, eye, and mesothelioma. Cases are identified through the rapid reporting system of the population-based MDCSS. Cases fitting study criteria are interviewed by telephone within 2 to 6 months after diagnosis. This brief interview takes an average of 11 minutes to complete and includes a health history; smoking history; complete occupational history including occupation, industry, and job description; residential history, and demographic characteristics. Analytical methods will include both PCIR and SIR.

Progress & Accomplishments

Through December, 1985, 5011 cases had been identified and entered into the study; 977 of these are in process. Of the 4034 completed cases, 3757 were interviewed and 277 were either refusals or non-interviews for other reasons. Thus, our current response rate for this study is 95.5%. Of those interviewed, 79.7% were white and 19.2% were black; 60.3% were male and 39.7% were female. To date, there have been 804 or 24.7% of cases who never smoked cigarettes. Specific analyses of these cases will be important in elucidating occupational risks, particularly for bladder and lung cancers and for mesotheliomas.

In addition to the interviews completed, all data have been entered into the computer and preliminary data analysis has been initiated. A specific computer process has been written for periodic screening and editing of the data. Significant effort has been invested in the development of an analytical scheme for grouping occupations and industries in terms of shared exposures that are potentially important for understanding occupational cancer etiology. An occupational physician consultant, Dr. Kelly Brix, has assisted with this task.

Significance

This project will develop the first population-based cancer incidence surveillance system in the United States which provides monitoring across a wide variety of occupations. Insights will be gained about occupational cancer risks among blacks and women and new leads are expected to be developed regarding the occupational etiology of cancer.

Publications

Swanson GM, Schwartz AG, Brown KL: Population-Based Occupational Cancer Incidence Surveillance--Utilization of the Telephone Interview. J of Occup Med 27:439-444, 1985.

FACTORS MODIFYING DIESEL PARTICULATE COMPOSITION

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Occupational Cancers
1 R01 OH02119-01
09/27/85 - 08/31/88
$69,031 ($69,031 Cum)

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Objectives

Ceramic particle traps are being developed for use as diesel exhaust emission controls for over-the-road as well as heavy-duty diesel engines used in mining. These devices collect entrained diesel aerosols, and on reaching the regeneration temperature, catalyze combustion of trapped materials. While they reduce the opacity of tailpipe emissions, and thus are desirable from an aesthetic point of view, little is known about the physical and chemical properties and biological activity of the particulates they transmit. Therefore, the possible impact of this new technology on human health needs evaluation. This is particularly true for occupational settings such as underground mines where workers may be exposed to diesel fumes at twenty-five times the levels found in congested urban canyons with proportionately greater risk.

The long-term project objective is to assess the relative hazard to human health of particulate emissions from diesel engines with and without ceramic particle traps. The study will focus on cancer risk as opposed to non-malignant respiratory effects.

The comparative risk assessment will be accomplished through the following specific aims: 1) collection of particulate samples from a commercial mining diesel under simulated mining conditions; 2) determination of the mutagenic potency of the diesel particulate soluble organic fraction (SOF) with the Ames *Salmonella*/mammalian microsome test; 3) definition of the operating modes which contribute most to the production of chemical carcinogens under steady-state sampling conditions; 4) further examination of the chemical composition and biological activity of particulate emissions under conditions of transient diesel engine operation.

Methodology

Diesel particle samples (provided by the U.S. Bureau of Mines) are collected from a diesel test cell consisting of a commercial diesel mining engine, dynamometer, an exhaust dilution tunnel and a particle collection system. The engine is operated in series of 6 modes, typical of mining equipment operated in a load-haul-dump cycle. After high dilution with filtered, pure air entrained particles are collected on Teflon-coated glass fiber filters. Thus, the particle samples collected simulate tailpipe emissions from machinery operating in underground mines.

It is generally accepted that bacterial mutagenesis assays alone do not provide sufficient information to index cancer hazards posed by particulate emissions. Thus, in this study the health hazard will be indexed by bacterial mutagenesis assay and chemical analysis of known carcinogens and mutagens.

Particle extracts are bioassayed with the *Salmonella typhimurium*/mammalian microsome assay, a widely-used short-term test which detects carcinogens as bacterial mutagens. The standard top agar incorporation assay will be used with the TA98 and TA100 strains which are sensitive to both frameshift and substitution mutagens. Metabolic activation assays will use the 9,000 g supernatant subfraction of Aroclor 1254-induced rat livers.

Chemical parameters to be determined in diesel particulate matter include 16 polycyclic aromatic hydrocarbons (PAH) and 10 nitro- and dinitro-substituted PAH (nitro-PAH). Many of the target analytes are carcinogens, but not all of the known carcinogens of diesel exhaust are represented. The PAH selected are markers or indicators of PAH content--many of the nitro-PAH also are animal carcinogens, but these structures account for the majority of the mutagenic activity in bacteria. Specific and sensitive trace measurement of these chemicals will be accomplished by a combination of chromatographic techniques including normal and reversed phase liquid chromatography with absorbance and floureecence detection and capillary gas liquid chromatography. The analytical chemical methodology developed in this project will be rigorously evaluated for selectively, accuracy and precision before use.

Progress & Accomplishments

Particle Generation and Sampling. The U.S. Bureau of Mines has collected complete sets (various loads and speeds) of particles from a commercial diesel engine. Sampling is ahead of schedule so that the impact of the ceramic particle trap, as well as a manganese-copper fuel additive have been tested. This additive improves the performance of the trap and thus may also find use in underground mines.

Mutagenicity of Particle Extracts. Particle samples from the mining engine with and without the trap have been tested for mutagenic activity and bioassays of fuel additive samples are in progress. Two trends are clear from this data: 1) the trap greatly reduces the mass of emitted particles (as claimed by the manufacturer) and 2) engines operated with the ceramic particle trap transmit particles with elevated mutagenic activity as hypothesized in our application for NIOSH support. Under baseline conditions (i.e., no trap) particles emitted have a mutagenic potency of 64 - 223 revertants/mg (mutagenic activity units)--with the trap in place the mutagenic activity ranges from 46 - 1,400 revertants/mg. The net effect of the properly operating trap, however, is to reduce the total output of mutagenic substances in the mine air (see Significance section).

Chemical Analysis. A sensitive and specific method has been developed and evaluated for measurement of nitro-PAH in diesel particulate matter and is being used to analyze particle samples collected this year.

Significance

The Ames test data have been incorporated in mathematical models developed by K. Baumgard and D. Kittelson, consultants to this project. These models extrapolate steady-state diesel test cell data to tailpipe emissions from mining equipment, and to pollutant concentrations in mine air. Such models predict that mass levels of inhalable diesel soot in poorly ventilated, underground mine air will be reduced by approximately 93% by the ceramic trap. Projected reductions in mutagenicity of breathing air (revertants/cubic meter) are approximately 83% when the machinery is fitted with the properly functioning trap. Based on the Ames test data alone, a sizable reduction in health risk is anticipated. Transient engine sampling (as opposed to steady-state) and chemical analysis of the emitted soot, however, are essential to adequately evaluate the health impact of the trap.

Publications - None to Date

URINARY EXCRETION OF MODIFIED NUCLEOSIDES

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Occupational Cancers
1 R01 OH02122-01
08/01/85 - 07/31/88
$94,504 ($94,504 Cum)

Objectives

Transfer RNA (tRNA) is a complex biomacromolecule containing a large number of modified bases. The modifications include methylation by several enzymes which are specific for bases, positions in bases, and specific for positions in tRNA. The most abundant of these enzymes are those which insert methyl groups at various positions in the macromolecule. The methylating enzymes have been found to be hyperactive in malignant tumor tissues, which therefore contain tRNAs that are different in structure from those present in normal tissues. These modified tRNAs also have a high turnover rate. It is known that patients with certain types of cancer excrete in their urine elevated levels of modified nucleosides (tumor markers). We have demonstrated abnormal levels of modified nucleosides in patients with asbestos related malignant mesothelioma. We have also shown, in a feasibility study, altered nucleoside excretion among individuals without clinical evidence of cancer but with no long-term history of occupational exposure to airborne asbestos. Measurement of modified nucleosides is therefore considered a possible approach to detection of a precancerous state or early diagnosis of cancer among individuals who are at high risk for developing a malignant disease. We are measuring levels of modified nucleosides in the urine of 1,000 asbestos insulation workers with a history of 30 years or more since first onset of exposure. The investigation focuses on whether the urinary excretion profile of nucleosides is related to the effects of asbestos exposure and whether the levels of urinary nucleosides may be predictive of subsequent malignant disease.

Methodology

We have obtained extensive clinical and laboratory information on 2,900 asbestos insulation workers from clinical field examinations performed between 1981-1983. A prospective mortality study has been designed, and the study population is currently kept under serveillance in terms of mortality experience. The nucleoside pattern will be characterized for the entire population, and a matrix of intercorrelations between neucleosides and pertient medical and laboratory information will be generated. The association between the initial nucleoside excretion pattern observed at the clinical field examination and the mortality experience will be studied.

Progress & Accomplishments

The 1,000 individuals who constitute the study population have been selected from the 2,900 examined in the clinical field studies. All were without history of cancer or clinical signs of malignant disease at the time of the examination. We are being notified about all deaths which have occurred in the study population. They are currently in the process of being ascertained, but earlier projections made in studies of this group of asbestos insulation workers seem to receive further confirmation.

Approximately 50 percent of the deaths are due to cancer, and the original observation of an extraordinarily high cancer mortality in this population will thus occur. Measurement of modified nucleosides in the first 500 urine samples is in progress. In preparation for the mortality analysis, expected mortality rates for the study population are being calculated by collaborating scientists at the American Cancer Society.

Statistical analysis is also proceeding with regard to characterization of the study population in terms of the baseline demographic, clinical and laboratory information which was obtained at the onset of the study.

Significance

The initial results of this project relate to the characterization of the study population and acquisition of mortality data. Our initial observations have shown that the study population experiences a high cancer mortality rate, similar to what has been observed in the large cohort of asbestos insulation workers from which the study population was derived. This provides us with a unique opportunity to obtain information about the predictive value of modified nucleosides in terms of the development of asbestos related neoplastic disease. The extensive amount of laboratory and clinical information available on this high risk population will also enable us to perform analyses with regard to multiple factor interactions.

Publications - None to date

LEUKEMIA RISK IN LINEMEN

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Occupational Cancers
1 K01 OH00031-01
09/28/84 - 12/31/85
$32,400 ($32,400 Cum)

Objectives

An epidemiologic case-control study is being conducted to assess the risk of leukemia in telephone linemen. The study tests the hypothesis that electric power lines mounted on poles shared between the electrical utilities and the telephone companies may represent a health risk to linemen servicing the telephone cables on these poles.

Methodology

Through an arrangement with the Medical Research Department at American Telephone and Telegraph (AT&T), all deaths from leukemia that occured among active or pensioned operating telephone company employees between 1975 and 1980 have been identified. Study cases include all active or pensioned employees who worked at least two years for AT&T, were identified through the AT&T mortality data base, died of leukemia in the six-year period 1975-1980, and did not have another underlying hematologic cancer. Specific cell types of leukemia have been identified from death certificates and company medical records. A matched control group has been selected from the Bell Systems Personnel Data Base. The controls are comparable to the cases in age, sex, race and calendar time of employment. Complete work histories for the study subjects have been obtained from telephone company personnel service record cards. Visits to telephone companies have identified specific job titles and activities associated with line work and potential exposure to electric and magnetic fields, and individual exposures will be estimated from jobs held and duration in the job. In the analysis, the proportions of cases and controls who were ever in jobs associated with field exposure will be compared.

Progress & Accomplishments

Copies of articles published since 1960 relevant to human health effects of 10-100 H_z electromagnetic field exposure (excluding direct electrical stimulation) have been obtained and reviewed. Abstracts are being written and added to the data retrieval system to create a complete, annotated bibliography. Articles on the human health effects of substances to which linemen may be exposed (such as asbestos, benzene, herbicides, insecticides, petroleum products, polychlorinated biphenyls, and soldiers) have been reviewed and selectively collected.

Four hundred and twenty-nine operating telephone company employees who had died of leukemia between 1975 and 1980 were identified. The names of these potential cases were submitted (prior to any exclusions) to the appropriate operating telephone company medical offices for confirmation of the death certificate diagnosis of those cases with medical records on file, additional information on cell type of leukemia, and identification of study subjects with another hematologic cancer that predated their leukemia. One hundred and thirteen individuals were excluded from the study because they had worked for less than two years (4), were diagnosed with a hematologic cancer that predated their leukemia (14), were nonwhite (7), hand chronic lymphoid leukemia (87), or both another hematologic cancer and chronic lymphoid leukemia (1). Individuals with chronic lymphoid leukemia were excluded because this form of leukemia is usually not rapidly lethal, and will be incompletely ascertained from death records such as the AT&T mortality data base. Photocopies of the death certificates for all cases but one have been obtained from the files at AT&T. A trained coder has coded these using the 1975 International Classification of Diseases. Three controls have been selected for the 316 cases by probability sampling, and are matched to cases in year of birth, year of hire, race, sex and company. The control sampling frame is the tape copy of the complete Bell System Personnel Data Base (1.2 million employees each year) for the years 1975-1980. The controls are individuals who have not died of leukemia and who were active or retired at the same time as the leukemia case.

The medical directors of each telephone company have identified a supervisor from personnel or payroll as a contact who would provide the work histories of the study subjects. We have requested work histories for the study subjects. We have also requested information on race, level of education, and jobs held prior to employment with the Bell System, when available. By December 31, 1985, we had received photocopies of work histories for 30% of the study subjects. Companies 23 and 40-42 have stated that they would require employee authorization to release work history information. Using information from personnel offices and from the actual work histories received, an alphabetical listing of Bell System occupations has been compiled. Jobs will be classified by appropriate categories and a coding scheme will be developed. Available records do not permit quantification of actual electromagnetic field exposures for each individual study subject. The most that is achievable is collection of work history data on months or years in jobs with potential exposures, and characterization of these jobs by current exposure levels. Site visits have been conducted at Chesapeake and Potomac Telephone and at Mountain Bell to obtain job descriptions for linemen and observe linemen at work rural and urban sites. Linemen can be categorized by the following job titles: installer-repairer, outside plant technician, cable splicer, and helpers who work with each category of lineman. A description of the common activities and work locations for these job classifications has been developed based on the interviews with supervisors and the review of job descriptions and daily work logs obtained from the telephone companies. A questionnaire has been developed to obtain an estimate of exposures other than electromagnetic fields by subgroups of linemen.

Significance

Recent reports have suggested that persons who are exposed to power lines are at increased risk of leukemia; this study will test that hypothesis. To date, it is our conclusion that an epidemiologic case-control study of leukemia in telephone company employees is feasible; however, since collection of work histories depends upon the cooperation of so many personnel departments, it is a source of more delay than originally anticipated.

Publications - None to date

ENVIRONMENTAL CHEMICALS AND EUKARYOTIC PROTEIN SYNTHESIS

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Occupational Cancers
5 R03 OH01795-02
07/01/83 - 06/30/85
$25,674 ($47,643 Cum)

Objectives

The specific objectives of this research proposal are:

1. To study the effect of benzene on the specific phosphorylation of eIF-2α in rabbit reticulocytes.

2. To purify the benzene-activated translational inhibitor(s) from rabbit reticulocytes.

3. To study the mode of action of the purified benzene-activated translational inhibitor(s).

Methodology

The following experimental approaches were used to study the effect of benzene on inhibition of protein synthesis in rabbit reticulocytes.

1. Increase of specific eIF-2α phosphorylation in unfractionated rabbit reticulocyte lysates. This goal was accomplished by the use of fructose 1,6-diphosphate, NAD^+, ^{32}Pi and an energy regenerating system that allows for the generation of high specific activity $[\gamma-^{32}P]ATP$ by glycolysis and results in the phosphorylation of initiation factors in situ. The increased phosphorylation of eIF-2 is accompanied by a reduction in the biological function of eIF-2, namely, the formation of a ternary complex between eIF-2, the nucleotide GTP and the initiator tRNA. These results conclusively demonstrate that benzene can activate a translational inhibitor in rabbit reticulocyte lysates.

2. Purification of benzene activated eIF-2α protein kinase. This goal was partly accomplished by chromatography of the reticulocyte postribosomal supernatant on DEAE-cellulose, phosphocellulose, hydroxyapatite, and Sepharose 6B.

Progress & Accomplishments

1. Benzene was shown to inhibit polypeptide initiation in rabbit reticulocyte lysates. This conclusion is based on (i) the inhibition of the transfer of formylmethionine from $f[^{35}S]$Met-tRNA into polypeptide, and (ii) the inhibition of the initial dipeptide, methionylvaline, accumulation in the presence of pactamycine.

2. Benzene specifically increases the phosphorylation of the α-subunit of initiation factor eIF-2. This was conclusively demonstrated by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

3. Benzene activates an inhibitor of protein synthesis in rabbit reticulocyte lysates. This inhibitor appears to be similar or identical to the heme-regulated translation inhibitor.

Significance

Data obtained in this two-year study clearly establishes that benzene influences the initiation step of protein synthesis in mammalian cells by activating a potent kinase. Because protein synthesis is tightly coupled to DNA synthesis and cellular differentiation; a lesion in translations initiation may be expected to adversely affect cell function by first modifying the DNA replication machinery. These effects may in turn partly account for the clinical symptoms of chronic benzene toxicity.

Publications - Abstract(s) available

PROSTAGLANDIN SYNTHETASE AND LUNG TOXICITY WITH BHT

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Occupational Cancers
5 R03 OH01833-02
07/01/83 - 12/31/85
$19,600 ($36,409 Cum)

Objectives

The major objectives of this study were to determine (1) whether butylated hydroxytoluene (BHT) is metabolized to a reactive intermediate by prostaglandin H synthase and (2) whether peroxidase activation of BHT is a significant pathway in the in vivo toxicity of BHT to mouse lung.

Methodology

Two model peroxidase enzymes, horseradish peroxidase and prostaglandin H synthase, were used for the studies of BHT metabolism. Progtaglandin H synthase was obtained from ram seminal vesicles. The in vitro formation of reactive metabolites was monitored by (1) the covalent binding of ^{14}C-radiolabeled BHT to protein, (2) the formation of BHT-quinone methide, the purported ultimate toxic metabolite of BHT, by spectorphotometric methods, and (3) the formation of free radicals from BHT using electron spin resonance techniques. Stable metabolites of BHT were identified by conventional methods. The in vivo toxicity of BHT to mouse lung was assessed by measuring lung weights and by histopathology four days after exposure.

Progress & Accomplishments

The major findings from the past year on this project can be summarized as follows:

A. BHT was converted by peroxidase enzymes to a reactive metabolite which covalently bound to microsomal protein. This reaction was concentration dependent and was inhibited by glutathione and certain peroxidase substrates. BHT also formed two polar metabolites (one aqueous, one organic) as detected by thin layer chromatography. The formation of these metabolites increased when glutathione was present suggesting they might be glutathione or protein conjugates.

B. In the presence of butylated hydroxyanisole (BHA), the covalent binding of BHT was enhanced (+400%). In addition, two additional products were detected: BHT-quinone methide and stilbenequinone. The formation of the highly reactive quinone methide might account for the increase in covalent binding.

C. In addition to BHA, several other compounds were capable of enhancing BHT covalent binding and quinone methide formation. These included compounds which were able to form phenoxy radicals or nitrogen-centered cation radicals. Alternatively, compounds which formed peroxy radicals or semiquinone radicals inhibited BHT metabolism. A direct chemical-chemical interaction between the BHA radical and BHT resulting in the generation of quinone methide was proposed to explain the enhanced metabolism and covalent binding of BHT.

D. A free radical resulting from the metabolism of BHT by horseradish peroxidase was detected using electron spin resonance and is currently being analyzed to determine the chemical structure of the radical.

E. Microsomes from various animal tissues (mouse lung, rat lung, human lung, dog lung, guinea pig lung, sheep lung, rabbit kidney medulla, and rat small intestine) were capable of catalyzing the covalent binding of BHT to protein using either arachidonic acid or hydrogen peroxide as substrates. BHA was able to enhance BHT binding in each case.

F. In the mouse lung, subcutaneous administration of BHA (50 or 250 mg/kg body weight) was observed to significantly enhance the toxicity of subthreshold doses of BHT (0-250 mg/kg) as measured by increased lung/body weight ratio. This enhancement of lung/body ratio was dependent on the time of administration of BHA and was much more effective when BHA was injected subcutaneously than intraperitoneally.

Significance

The results presented here demonstrate an interesting post-enzymic interaction between BHA and BHT resulting in the formation of a toxic metabolite, quinone methide. This method of activation of BHT by a chemical-chemical interaction represents a method of activation of a xenobiotic compound for which there are few examples in the literature. This interaction between BHA and BHT can take place in a variety of animal tissues which contain peroxidase enzymes, including human lung. In the mouse lung, BHA was also able to enhance BHT-induced lung toxicity. These results suggest that tissues which contain peroxidase enzymes might catalyze the formation of the toxic quinone methide. These results also point the importance of considering toxic effects from combinations of chemicals that are commonly used together in addition to testing them singly.

Publications - None to date

SILICA, SILICOSIS, AND CANCER: AN INTERNATIONAL SYMPOSIUM

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Occupational Cancers
1 R13 OH02086-01
04/01/84 - 11/30/84
$10,000 ($10,000 Cum)

Summary

The University of North Carolina at Chapel Hill and the Society for Occupational and Environmental Health jointly sponsored an international symposium entitled "Silica, Silicosis, and Cancer". The Symposium was held April 3-5, 1984, in Chapel Hill, North Carolina.

The rationale for convening this symposium is that there is now evidence that silica may act as a carcinogen. Silica has heretofore been considered a fibrogenic dust that causes silicosis, the sequelae of silicosis, and an old occupational disease whose incidence is declining. If silica may be a carcinogen (alone or combined with other hazardous materials), the concern is that there is a range of between 520,000 and 3,200,000 workers potentially exposed. Some of the occupations include auto workers, cement workers, ceramic workers, construction workers, foundrymen, glass makers, granite workers, metal ore miners, masons, quarrymen, sandblasters, silica brick workers, and tunnel builders.

Recent experimental animal evidence suggest that silica alone produces pulmonary histiocytic lymphomas and lung tumors. These experimental results support the findings of lung and gastrointestinal cancer excesses observed among workers who it is assumed were highly exposed to silica and other particulates. Despite the general lack of industrial hygiene measurements to confirm the effect of silica exposure, lung cancer excesses have been reported for the following occupations: gold and metal ore miners, foundry workers, sandblasters, silica firebrick makers, ceramicists, and granite workers. Miners, foundrymen, and granite workers also have elevated risks for gastrointestinal cancers. Of special note are the striking relative risks between three and six for lung cancer in two followup studies of silicotics in Sweden and Canada. Swedish researchers have recently confirmed their initial result with a new case-control study of lung cancer among silicotics.

Leading researchers in this field addressed the Symposium. In addition, presentations of new findings were made. We anticipate that the Symposium and publication of the proceedings will permit scientists in this wide field to share common intellectual ground, thus permitting new research endeavors to be undertaken.

Publications

Silica, Silicosis, and Cancer: Controversy in Occupational Medicine. Goldsmith DF, Winn DM, Shy CM (eds), Praeger Publishers, New York, 1985.

EXPLOSION HAZARDS RELATED TO GRAIN AND FEED DUSTS

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Traumatic Injuries
5 R01 OH01122-05
01/01/81 - 07/31/87
$106,384 ($480,885 Cum)

Objectives

The purpose of this research project is to quantitatively characterize the explosion hazard represented by suspended and layered combustible dusts. Although much explosion testing with combustible dusts has been done for many years, almost all of these results are apparatus dependent. The results fail to characterize a fundamental physical quantity, and none of it directly pertains to layered dust hazards. Under this project the burning velocity for a suspended dust cloud and for a layer of dust is being measured. Appropriate analytical efforts are also required in order to understand the effect of several physical variables -- composition, concentration, size, moisture content, shape, volatility, etc.

Methodology

It was necessary to develop two new pieces of equipment in order to avoid the criticisms associated with previous dust explosion testing. The suspended dust combustion studies are conducted in the Premixed Turbulent Combustion Bomb (PTCB), which is a spherical, one cubic meter jet stirred reactor. The combustion process is initiated at the center of the well characterized, uniform, turbulent dust cloud and during its propagation toward the vessel wall appropriate measurements are made to characterize the burning process. The layered dust testing is done in the Flame Acceleration Tube (FAT), which consists of three continuous segments of 3000 psi working pressure steel tubing each having a length of forty feet and an inside diameter of one foot, closed at one end and open at the other. A controlled thickness and width dust layer may be placed along the bottom of the tube which is then ignited by the combustion of a presuspended dust cloud in the first twelve feet at the closed end. The history of the subsequent combustion process as it accelerates toward the open end is monitored using appropriate instrumentation. Recently two significant improvements have been made to this facility -- steel blast mats are used to enclose the open end, thus attenuating the air blast, and a CAMAC digital data acquisition system is used to control the run and to acquire and reduce the data. In both facilities, for different dust conditions, the flame propagation and properties are characterized.

Progress & Accomplishments

Using the PTCB the laminar and turbulent burning velocities have been measured for two combustible dusts -- mixed grain dust and cornstarch, as well as the flame thickness. Similar work has just been completed for methane/air mixtures. Using the FAT both the burning and the flame velocities have been measured as well as the accompanying overpressures and the conditions necessary for propagation for a wide variety of combustible dusts. Additionally, a computer code has been employed which with proper specified conditions predicts the flame behavior.

Significance

For a suspended dust/air mixture the turbulent burning velocity is a fundamental physical parameter. Knowledge of the quantity allows analytical prediction of flame propagation as it would occur during the course of an accidental explosion. For the layered dust the minimum hazardous thickness is the quantity of significant interest. Housekeeping which maintains lesser accumulations should preclude the occurrence of accidental explosions. And, when the entrainment mechanism is understood the suspended dust situation may then be merged with the layered dust problem, thus too allowing analytical predictions.

Publications

Kauffman CW: Agricultural dust explosions in grain handling facilities. SM Study #16, University of Waterloo Press, 1982.

Kauffman CW, Nicholls JA: Dust explosion research at The University of Michigan. SM Study #16, University of Waterloo Press, 1982.

FEEDBACK AND SAFETY: INVOLVING WORKERS

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Traumatic Injuries
5 R01 OH01928-02
12/01/83 - 11/30/85
$30,347 ($92,718 Cum)

Objectives

The basic purpose of the study was to attempt to determine the extent to which involving workers in a program of safety might enhance safe performance on the job.

The specific objectives were:

1. To revise and update a pool of safe practices and conditions by using the assessment strategy described in Sulzer-Azaroff and Fellner (1984).
2. To establish baselines for work units by measuring those practices and conditions for time periods of different lengths.
3. To implement the standard feedback system, as described in Sulzer-Azaroff (1978), Sulzer-Azaroff and de Santamaria (1980) and Fellner and Sulzer-Azaroff (1984).
4. Then to involve peers by selecting volunteer members of each work unit and training them to conduct safety audits within their units.
5. Independently to continue monitoring safe conditions and practices to determine if peer involvement appeared to enhance or sustain safe practices.
6. To monitor accidents and lost time injuries during each phase, as well as workers' perceptions of the safety climate in the plant as compared with those in a control plant in which the interventions were not implemented.

Methodology

All the employees in 21 work units of a paper mill in Massachusetts were involved. Safety targets, performances and hazardous conditions, were identified for each unit. Baselines were collected for varying lengths of time for each unit. Following baselines, each unit identified 3 safety targets on which to work in sequence and feedback was provided on those targets by the experimenters. In 12 units peer volunteers were then trained to measure the targets and post results, which they continued for varying lengths of time.

Safety targets for each unit were observed on multiple occasions within each phase and follow-up observational data recording continues. A Safety Climate Questionnaire was administered once to a control plant and twice at the experimental plant.

Progress & Accomplishments

When feedback was provided on safety targets, performance improved to levels of statistical significance. During peer feedback, improvement diminished slightly but still remained significantly higher than during baseline. Results of the Safety Climate Questionnaire revealed no differences. Follow-up collection of data on safety and on accidents is ongoing.

Significance

The fact that it was possible to sustain improved performance when the feedback function was transferred to peers is most encouraging. Assuming that an organization is set up to promote continued peer involvement, one can anticipate that improvement in safety performance can be helped to sustain via that method.

Peer mediated feedback is cost efficient and non-intrusive. It should sensitize involved workers to the hazardous conditions in their workplace with little extra time and effort. It is hoped that this will eventually lead to reduced rates of accidents.

Publications - None to date

WORK AND RISK OF CARDIOVASCULAR DISEASE IN THE FRAMINGHAM STUDY

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Cardiovascular Effects
1 R01 OH02057-01
01/01/85 - 12/31/85
$121,342 ($121,342 Cum)

Objectives

To carry out a prospective examination of the incidence of cardiovascular disease in relation to long-term work patterns based on 30 years of surveillance of the Framingham Study cohort. To classify subjects according to employment status, work environment, employers, and to merge these work history data with other cardiovascular risk factor data, demographic and social variables and cardiovascular disease status. From this data to determine the incidence of cardiovascular disease in relation to the work history variables and to assess the net and joint effect of the work variables on cardiovascular disease incidence taking into account the major associated cardiovascular risk factors. Interactions between work history variables and other demographic and psychosocial variables are to be explored in relation to impact on cardiovascular morbidity and mortality.

Methodology

The effects of labor force participation, occupations and employers on cardiovascular outcomes such as coronary events, strokes, cardiac failure and peripheral arterial diseases is examined taking into account medical, psychosocial and demographic variables using multivariate multiple logistic regression analysis. This is done prospectively using DOT work classification codes at each biennial exam in relation to occurrence of new cardiovascular events over the 2-year interval. The relation of work variables to cardiovascular risk factors is examined including: blood lipids, blood pressure, blood sugar, cigarette smoking, relative weight and ECG abnormalities. Using multivariate analysis the effects of work variables on cardiovascular morbidity and mortality is examined taking into account the major associated cardiovascular risk factors.

Progress & Accomplishments

The classification and coding of work variables including: unemployment history, number of jobs held, part-time work, overtime work, physical demand of job, psychological stress, DOT and census occupational categories and exposures to noxious agents has been completed for each biennial exam over 30 years of follow-up on 5209 men and women participating in the Framingham Study. Work tapes including these variables, cardiovascular risk factors and cardiovascular outcomes have been created. Preliminary analysis relating these work variables to cardiovascular outcomes has been carried out identifying a number of positive findings justifying further, more detailed analysis.

Significance

Epidemiologic investigation indicate powerful environmental influences on cardiovascular risk factors and on cardiovascular events. These data provide information on the work environment, a major component of life-style which is modifiable. Knowledge of occupational exposures as risk factors for cardiovascular disease is of great preventive importance.

Publications - Abstract(s) available

LEAD BURDENS, EXPOSURE ESTIMATES AND HYPERTENSION

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Cardiovascular Effects
5 K01 OH00011-02
09/28/84 - 08/31/86
$19,440 ($51,840 Cum)

Objectives

The two major objectives of this project are to develop epidemiologically useful tools for estimating cumulative lead exposure and to conduct a pilot case-control study investigating a potential relationship between lead and hypertension.

Methodology

Standard twenty-four hour diagnostic chelation was conducted on a group of patients with and without known lead exposure. These patients then underwent x-ray fluorescent determination of skeletal stores of lead at the distal ulna (styloid process), and at the temporal bone. Correlation between XRF values and excreted lead was determined. Sensitivity and specificity of XRF vis a vis standard diagnostic chelation was determined using 600 mcg Pb/24 hour as the cut-off point.

Fifteen patients with and without known lead exposure received standard 24 hour diagnostic chelation for lead. Volumes and lead concentrations were additionally measured at three hours and at six hours lead excretion at three and six hours was correlated with total 24 hour results. Five additional patients have been recruited to bring the total to 20.

An exposure questionnaire has been developed and pretested. Composite lead estimates and individual questions will be validated for patients selected without knowledge of exposure status by using results of lead excretion.

Twenty randomly selected hypertensive patients are being matched with twenty controls on the basis of age, sex, race and economic status. A pilot case-control study will compare lead excretion using six-hour chelation results and questionnaire results.

Progress & Accomplishments

The x-ray fluorescent determination of skeletal stores at the wrist was found to have a correlation coefficient of .52 with 24 hour urine lead excretion following standard chelation ($.05 > p > .02$).

Specificity was 100% but sensitivity was only 56% using 600 mcg Pb/24 hour as the cut-off, and false-negatives occurred in individuals with lead excretion as high as 2500 mcg/24 hour. Results from XRF determinations as the temporal bone showed even less epidemiologic screening.

Three hour lead excretion following chelation has a correlation coefficient of .87 compared with standard 24 hour results. Six hour lead excretion has a correlation coefficient of .97 with 24 hour results. Both relationships are statistically significant and appear to be linear. We are collecting a total of 20 subjects and plan to submit this method for publication in the near future (an abstract has already been submitted to SREPCIM).

Both questionnaire validation and the pilot case-control study are proceeding using the six-hour urinary lead collection.

Significance

The study to date has validated a shortened form of diagnostic chelation which is feasible for use as an outpatient procedure. This can be used as an epidemiologic tool for case-control studies evaluating the chronic effects of cumulative lead exposure, one example of which is the pilot study of hypertensives and non-hypertensives being conducted. Similar studies of patients with dementia, gout, or cerebrovascular accidents could use this tool.

Publications - Abstract(s) available

A STATISTICAL METHOD FOR DETECTION OF WORK-RELATED ASCVD

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Cardiovascular Effects
5 R03 OH01867-02
07/01/83 - 06/30/85
$13,194 ($26,777 Cum)

Objectives

In the analysis of occupational cohort mortality data, if workers with disabling illnesses terminate employment early, standard methods that estimate mortality as a function of lifetime cumulative exposure tend to underestimate the true effect of exposure on mortality, whether or not one adjusts for time of termination of employment. The degree of underestimation will be greatest for mortality from chronic disabling illnesses such as arteriosclerotic cardiovascular disease. Underestimation of the effect of cumulative exposure can lead to reduced power to detect new occupational causes of disease that fail to adequately protect workers. The objective of this grant was to develop a statistical method to control for the "healthy worker survivor bias" resulting from the early termination of disabled workers.

Methodology

Our approach to controlling the healthy worker survivor bias was to identify the observed occupational cohort mortality study with a "hypothetical double blind randomized trial" in which data on assigned treatment protocol had been erased from the data file. A worker was assumed to be "on protocol" while at work, but to be "off protocol" when off work. In a double blind randomized trial with data on treatment protocol missing from the data file, the causal association of mortality with (the missing) treatment protocol can still be estimated when data on date of death, observed exposure history and date of leaving and returning to treatment protocol are available. Thus we are able to estimate the causal association of mortality with the treatment protocol of the hypothetical randomized trial.

Progress & Accomplishments

We developed statistical software to estimate the survival-treatment protocol associations of the hypothetical double blind randomized trial associated with any cohort mortality study. In addition, we developed a new nonparametric test of the null hypothesis of no mortality treatment protocol association, and developed the software to implement it. We reanalyzed, with our method, data from a large mortality study of Montana copper smelter workers exposed to arsenic. For workers first hired past 1935 (after exposures had been lowered), we were able to detect significant adverse effects on arsenic exposure on total mortality and lung cancer mortality even though standard methods failed to detect any dose-response relationship. We were unable to find statistically significant effects of arsenic exposure on heart disease mortality, although there was a nonsignificant increase in the risk.

Significance

Our results show that small to moderate increases in the relative risk (in the range of 1.1 to 2.0) due to occupational exposures can be masked by the healthy worker survivor effect. Since present-day exposures are, in general, lower than exposures experienced in the past, and since most substances associated with large increases in relative risk may have already been discovered, occupational epidemiology is becoming increasingly focused on detecting relative risks in the range of 1.1 to 2.0. Our method appears able to detect these small increases in risk even when standard methods are unable to do so.

Publications - None to date

HEART DISEASE IN METALWORKERS: OCCUPATIONAL DETERMINANTS

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Cardiovascular Effects
1 R03 OH01904-01
05/01/84 - 04/30/85
$7,095 ($7,095 Cum)

Summary

This proposal will investigate the etiological relationship of noise, heat, and other hazards in the work environment to cardiovascular disease and overall physical health status.

The data used will be from the METELLI project, a prospective ten-year study on metalworkers in Finland that has collected information in exposures to specific hazards, job characteristics, psychosocial functioning, and medically-determined health status.

Independent variables of interest to this study include industrial hygiene measurements of noise, heat, silica, and light; job characteristics such as work pace, monotony; psychosocial functioning such as social support, stress symptoms; and health practices such as smoking and alcohol consumption, diet.

Outcome measures will include cardiovascular disease morbidity and mortality and overall morbidity and mortality.

Results will provide evidence on the individual and joint effects of the independent variables on worker health, with particular attention to cardiovascular disease.

Publications - None to date

OCCUPATIONAL RISKS OF PESTICIDE EXPOSURE FOR FEMALES

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Reproductive Disorders
2 R01 OH00835-07
09/15/85 - 08/31/88
$59,864 ($279,333 Cum)

Objectives

The present project was designed to characterize some of the reproductive hazards that confront females engaged in occupations which subject them to potential exposure to pesticides. The major thrust of the past year was directed toward ascertaining whether Kepone, a chlorinated insecticide, could induce an alteration in the ovulatory response to exogenous gonadotropins and whether such a response would be reversible. In addition, the effects of this pesticide on normal body weight, liver weight and incorporation of said pesticide into the liver were also to be evaluated.

Methodology

Virgin female CD-1 mice were randomly distributed into three major treatment groups: a Kepone-treated group, a group treated with estradiol-17β(E-17β) and sesame oil vehicle control group. Mice were exposed to these agents for either two or four weeks. Weekly procedures consisted of five consecutive daily exposures followed by two days of no treatment. This time table was established to mimic an ordinary five day work week which would represent the maximum weekly exposure to which a female working with such a compound might be subjected. Kepone (0.25 mg), estradial-17β(0.1 mg) and sesame oil were administered in a 0.2 ml volume by oral gavage. During the second and fourth weeks exogenous gonadotropins (PMSG and HCG) were administered to determine whether the ovaries could elicit an ovulatory response to these gonadotropins. At the end of the two and four week periods, the animals were sacrificed. Livers were removed from the mice, weighed and prepared for gas chromatographic analysis. Oviducts were also removed from the mice and flushed to remove ovulated ova.

Progress & Accomplishments

Results revealed a significant decrease ($P<0.05$) in the number of ovulated oocytes in animals treated with Kepone for four weeks when compared to E-17β treated animals and sesame oil controls. In order to determine if this decreased ovulatory response was reversible animals were removed from the Kepone regimen and allowed to feed normally for three weeks. During this third week they were subjected to exogenous gonadotropins. Results revealed no significant difference in the ovulatory response among all three groups. In addition, the data indicated that exposure to 0.25 mg Kepone for as little as two weeks as % of total body weight. The accumulation of Kepone within the liver reached its maximum within two weeks increased this incorporation minimally. Incorporation decreased following withdrawal of the pesticide for three weeks.

Significance

Kepone induces a reduction of the normal ovulatory response even in the presence of gonadotropic stimulation although this effect is reversible once Kepone exposure ceases. It is not known whether the small number of ova ovulated by Kepone-exposed mice are viable and able to be fertilized.

Publications - Abstracts available

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SPERMATOGENIC TRANSFER OF THE EFFECTS OF MUTAGENS

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Reproductive Disorders
5 R01 OH01245-03
03/01/82 - 02/28/85
$62,548 ($176,223 Cum)

Summary

There is considerable concern about identifying the potential risks for individuals who work in occupational settings requiring frequent exposure to chemicals which may have mutagenic or carcinogenic potential. While women of child-bearing age are frequently not permitted to work in these high risk settings, men are not so protected. The teratological effects of *in utero* exposure to toxic agents is fairly well studied but the potential mutagenic effects on the male have just begun to be appreciated. We propose to examine the mutagenic effects of a number of chemicals which are felt to present significant risks to man. Our approach is to utilize a rodent model to examine the spermatogenic transfer resulting from the treatment of the male parent with selected mutagens to the F1 progeny. Our studies include the examination of behavioral development in the progeny, cytogenetic studies for chromosomal abnormalities and neuroanatomical and neurochemical examination of the brains of the progeny. In addition, we will examine mid-gestation embryos in order to determine pre- and post-implantation mortality and embryonic chromosomal characteristics. We plan to breed a portion of the F1 progeny and determine if evidence of behavioral teratogenesis, cytogenetic anomalies or neurochemical alterations exist in the F2 generations as a result of mutagen exposure.

Publications

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EMBRYO CULTURE/TERATOGEN BIOTRANSFORMATION SYSTEM

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Reproductive Disorders
5 R01 OH01281-03
09/29/82 - 08/31/85
$114,466 ($309,960 Cum)

Objectives

Enzyme inducing agents, including phenobarbital, 3-methylcholanthrene, polychlorinated biphenyls, ethanol (and others, if time permits) will be employed to investigate the qualitative and quantitative effects of changes in enzyme complement on malformations elicited in the embryo culture systems. These inducing agents will be studied in Sprague-Dawley rats during the earlier stages of gestation and effects as various xenobiotic biotransforming systems will be assessed to better determine what relationships exist between metabolic activities and incidence and type of observed malformations. The relative effects of tissue homogenates, slices, various homogenate subfractions and fine tissue fragments can help us to better evaluate the contribution of the various participating enzymes.

Methodology

In cases in which bioactivation of proteratogens have been established in the embryo culture system (e.g. with 2-acetylaminofluorene), attempts will be made to identify proximate and ultimate teratogenic species. A number of approaches will be utilized in attempts to accomplish this goal. Suspect metabolites can be added directly to the embryo culture system; selective inhibitors and activators of specific metabolic pathways can be used to corroborate other evidence; free radical scavengers, antioxidants, inhibitors of lipid peroxidation, etc., may also be found useful in determining not only which metabolite or metabolites may be acting as proximate or ultimate teratogens in vitro, but also through what mechanisms the reactive intermediates may be eliciting the malformations.

Progress & Accomplishments

In an initial study, we demonstrated for the first time that an exogenous source of xenobiotic biotransforming enzymes (hepatic 9000g supernatant fraction (S9) could be cocultured successfully with whole rat embryos. We also demonstrated that an exogenous bioactivating system (rat S9) was essential for the conversion of cyclophosphamide to a teratogenic intermediate and that the bioactivating reaction was cytochrome P-450-dependent. In other published investigations, we utilized the whole embryo culture system to study the role of biotransformation on the capacity of several other teratogenic agents to elicit malformations in cultured embryos. These included cytochalasin D, salicylate, 2-acetylaminofluorene, adriamycin, niridazole, and diethylstilbestrol. A principal advantage of the embryo culture system is that it permits evaluations of chemically-elicited dysmorphogenesis in the absence of complicating maternal factors.

Early experiments in our laboratories convincingly demonstrated that cytochrome P-450-dependent systems (added as S9 fractions with cofactors) could markedly affect the capacity of chemicals to elicit malformations. For some chemicals -- e.g., cyclophosphamide and 2-acetylaminofluorene -- a P-450-dependent system was requisite to chemical dysmorphogenesis due to the

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necessity for bioactivation. For cytochalasin D niridazole, it could be demonstrated that P-450-dependent biotransformation could play a major determining role in their teratogenicity via metabolic inactivation. For salicylate and diethylstilbestrol, exogenously added biotransformation systems appeared to play only a minor or no role as a determinant of teratogenicity in vitro. An added P-450-dependent system appeared to enhance the capacity of adriamycin, fifampicin and busulfan to produce embryotoxic effects even though these agents were active without such additions. In unpublished preliminary studies, we have also found that the teratogenicity of procarbazine and aflatoxin B_1, can be markedly influenced by biotransformation/bioactivation factors.

Significance

Recent research data from our laboratories have strongly indicated that rat embryos at days 8-10 of gestation can respond significantly to certain inducers of P-450 cytochromes. When cultured with a proteratogenic agent, 2-acetylaminofluorene, the conceptuses exhibited the capacity to convert the proteratogen to sufficient quantities of reactive intermediates to elicit grossly observable morphologic abnormalities in the same cultured (target) embryos. These observations imply that control of embryotoxic responses to certain chemicals can reside within the conceptus per se and opens a host of important questions that should be answered with research.

Publications

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Faustman-Watts EM, Yang HYL, Namkung MJ, Greenaway JC, Fantel AG, Juchau MR: Mutagenic, cytotoxic, and teratogenic effects of 2-acetylaminofluorene and reactive metabolites In Vitro. Teratogenesis, Carcinogenesis, and Mutagenesis 4:273-283, 1984.

OCCUPATIONAL EXPOSURE OF PARENTS AND TUMORS IN OFFSPRING

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Reproductive Disorders
5 R01 OH01413-03
02/01/82 - 01/31/85
$189,478 ($475,561 Cum)

Objectives

The objectives of this research are to determine the extent to which occupational exposure of parents accounts of tumors in the offspring and to determine the extent to which other environmental exposures of parents and children convey a risk for childhood tumors.

Methodology

The research projects employed a case-control approach focusing on childhood brain tumors and leukemias. Cases came from the Los Angeles County Cancer Surveillance Program (CSP), a county-wide tumor registry. After cases were identified (less than 10 years old), controls were selected from friends of the cases and controls were interviewed ascertaining a complete occupational and environmental history for each. Additional information was collected from the mother about the child.

Progress & Accomplishments

Central Nervous System Tumors - Work on the original grant has provided evidence that solvent exposure to fathers is an important factor for children's CNS neoplasms. It appears that methyl ethyl ketone, freon and trichloroethylene are three specific solvents that produce this risk. See the following table showing these risks:

Solvent Exposure in Fathers of Children with Brain Tumors

	12 CNS	10 Controls	Odds ratio
Solvents at all	12	7	∞
Daily Solvents	11	6	7.3
Carbon tetrachloride	3	1	3.0
Trichloroethylene	5	0	∞
Methyl ethyl ketone	7	1	12.6
Toluene	2	0	∞
Freon	4	0	∞
Paints	3	1	3.0
Petroleum Products	10	6	3.3
Asbestos/Fiberglass	4	3	1.1

By examining the table it can be seen that a remarkable number of CNS fathers work with solvents. The odds ratios vary from 3 to infinity with striking findings being related to trichloroethylene (5 to 0), methyl ethyl ketone (7 to 1) and freon (4 to 0) all of which are commonly encountered industrial substances.

Occupational exposures of the father to chlorinated hydrocarbon solvents, spray paint, dyes and pigments, methyl ethyl ketone, and cutting oils after birth of the child were associated with a significantly elevated risk of childhood leukemia. Exposure to chlorinated hydrocarbons was the only exposure of the father to remain statistically significant when adjusted for exposure to each of the other four significant exposures. During the pregnancy with the child, only the father's exposure to spray paint remained statistically significant (p=.023). Before the conception of the child, none of the father's occupational exposures were statistically significant. The father's exposures to aromatic solvents and radiation were similar in cases and controls.

As to the frequency of exposure to chlorinated hydrocarbons, case fathers were exposed more, on average, than control fathers. There was a statistically significant test for trend across zero, low (less than once/week) and high (more than once/week) frequencies of exposure to spray paint (p=.022). When looking at the frequency of the father's exposure to chlorinated hydrocarbons and methyl ethyl ketone, tests for trend had borderline significance (p=.062, p=.055).

The only statistically significant occupational exposure of the mother was to synthetic fabric after the birth of the child. The statistically significantly elevated risk remained when adjusted for the father's exposure to chlorinated hydrocarbons, and the reverse was true.

After adjusting for the five statistically significant exposures of the father, job titles and type of industry were examined. Children whose fathers ever worked as assemblers had an elevated risk of leukemia (p=.024), but the risk did not remain statistically significant after the birth of the child. Children whose fathers worked in the transportation equipment manufacturing industries, the majority being employed in the aircraft industry, after the birth of the child had an elevated risk of childhood leukemia (p=.053). Most of this elevated risk was due to exposure of miscellaneous or unknown solvents. Job titles when grouped into hydrocarbon related or electrically related occupations were not associated with statistically significantly elevated risks.

When examining non-occupational exposures, it was found that if either parent used household pesticides or burned incense in the home, during pregnancy or the nursing period, the child had a statistically significantly elevated risk of leukemia. These risks were independent of each other and the father's occupational exposure to chlorinated hydrocarbons and the mother's exposure to synthetic fabric. The father's occupational exposure to chlorinated hydrocarbons remained statistically significant when adjusted for the home use of pesticides or incense. The mother's occupational exposure to synthetic fabric, however, did not remain statistically significant when adjusted for these home exposures.

There were no other significant associations with the other diseases of the child or parents. Exposure of the child to x-rays or ultrasound in utero, or x-rays postnatally was not associated with a statistically significantly elevated risk. Parents' smoking and drinking habits, and the mother's medications and diets during pregnancy were similar in cases and controls.

Significance

Little is known about the etiology of childhood tumors. These studies have pointed to several different factors which offer the possibility of prevention of these devastating diseases.

Publications - None to date

THE ROLE OF HYPERTHERMIA IN CONGENITAL MALFORMATIONS

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Reproductive Disorders
5 R01 OH01434-03
09/29/82 - 08/31/85
$108,702 ($293,218 Cum)

Objectives

To determine the mechanism by which maternal hyperthermia during critical states of embrogenesis enhances the teratogenic effect of heavy metals.

Methodology

We have used the osmotic minipump as a method for the chronic continuous administration of heavy metals to pregnant animals. These pumps have been implanted subcutaneously. We have devoted most of our initial studies to the metalloid, arsenic. Levels of arsenic in the maternal blood, and in the placento-embryonic unit have been determined and correlated with the teratogenic end-point which in the case of arsenic are neural tube defects.

Progress & Accomplishments

Hamster dams dosed continuously with arsenate and exposed to short-term hyperthermia produced a greater percentage of malformed offspring than could be accounted for on the basis of the additive effects of the treatments. This synergistic response was enhanced by increasing the dose of arsenate. Heat treated hamsters possessed elevated arsenic concentrations in the maternal blood and the placentas immediately after cessation of the hyperthermic insult. Arsenic concentrations remained elevated in placentas for several hours, the duration being dependent on the dose of arsenate administered. The rise in placental arsenic concentrations may well be the basis of the arsenate/hyperthermia synergism in the production of fetal malformations in hamsters.

A manuscript of this subject has been submitted to Teratology and reprints will be forwarded when available.

In addition we have studied the effect of maternal hyperthermia on cadmium teratogenesis. The protocol for the experiment was as follows: Pregnant hamsters were injected (intraperitoneally) with an optimal dose of cadmium sulfate (2.0 mg/kg) at 8:00 a.m. on the eighth day gestation. Thirty minutes later one group of hamsters was placed in a water-jacketed incubator maintained at 39.5'C for a period of 45 minutes. Previous work by ourselves has shown that this thermal exposure elicits a minimal tratogenic response, and elevates the maternal rectal temperature from a normal of 37'C(+0.2') to an average of 41'C at the conclusion of the exposure. A second group of cadmium treated animals was injected with demineralized water only at 8:00 a.m. and after 30 minutes were exposed to heat for 45 minutes. The animals were killed by CO_2 inhalation on day 13 of pregnancy (5 days after treatment) and the fetuses recovered and examined for fetal malformations.

The Table summarizes the effect of the three treatments on the embyryonic resorption and malformation rate. Specific instances of a teratogenic synergistic effect can be shown in a four-fold increase in facila malformations in Cd-heat treated animals as compared to the two other groups, and a six-fold increase in encephaloceles (a neural tube defect) in the same relationship. The overall malformation rate is increased from 41% in the Cd-only treated group to 64% when the mother is exposed to an environmental hyperthermic event.

A manuscript on this data is in preparation.

Effect of maternal hyperthermia on cadmium-induced teratogenesis.

Teratogen	No. of Mothers	No. of Sacs	No. of Resorptions (%)	No. of Normal Fetuses (%)	No. of Malformations (%)
H_2O and Heat	15	226	17 (7.5%)	201 (96.2%)	8 (3.8%)
$CdSO_4$ Only	17	235	92 (39.1%)	84 (58.7%)	59 (41.2%)
$CdSO_4$	18	271	109 (40.2%)	58 (35.8%)	104 (64.1%)

Significance

There is now accumulating evidence that environmental hyperthermia (workplace, recreational), or endogenous fever, may result in an increased incidence of congential malformations. Our data show that one mechanism for this effect is that the hyperthermia increases the blood level of potential teratogens. We have shown this to be the case with respect to chronically administered arsenic. Acute cadmium teratogenesis is also enhanced by maternal hyperthermia. The exact mechanism of this synergism is as yet unknown, but increased maternal blood levels of the offending teratogen may result form the hyperthermic response.

Publications

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Williams KJ, Ferm VH, Willhite CC: Teratogenic dose-response relationships of etretinate in the golden hamster. Toxicology & Appl Pharm 4:977-982, 1984.

REPRODUCTION HAZARDS OF DINITROTOLUENE TOLUENEDIAMINE

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Reproductive Disorders
5 R01 OH01520-02
12/01/83 - 11/30/86
$166,549 ($308,811 Cum)

Objectives

The general goals of this study have been the same as defined in the original application, i.e. to define the anti-fertility effects of 2, 4-DNT (DNT) and the reproductive toxicity of 2, 4-TDA (TDA) in male rats.

Methodology

Groups of adult male rats were fed varying amounts of compound in their diet for 10 weeks or were placed on normal chow for an additional 11 weeks. Fertility was tested by mating males with receptive females and determining breeding ability. The reproductive performance of male rats were evaluated by determining mating, fertility, and viability indices. (i.e. # of sperm-positive females/# of exposed females per group, # of pregnant females/# of sperm-positive females per group, and # of live fetuses/# of implantation sites). The effects of exposure to the agent were assessed further, after sacrifice of males, at 10 weeks and at 11 weeks post TDA treatment by measuring serum LH, FSH, testosterone, epididymal sperm counts, reproductive organ weights, and by examination of the cellular architecture of the seminiferous tubules.

Progress & Accomplishments

We have established that diets containing 0.03% TDA fed ad libitum to male rats for 10 weeks results in decreased mating frequency and an increase in infertile matings. Light microscopic examination of the testes from these animals revealed reduced numbers of sperm in the seminiferous tubules and cauda epididymides. No effect on the number of viable implants or resorptions was found among pregnancies from males exposed to this dose or to lower dose levels of TDA.

Treatment with 0.03% of TDA for 10 weeks reduced the weight of the seminal vesicles and epididymides, reduced serum testosterone levels, and decreased cauda epididymal sperm counts. The reduced epididymal sperm reserves persisted in the TDA-treated animals placed on normal diet for 11 weeks. Furthermore, serum luteinizing hormone concentrations were increased and weights of epididymides and testes were reduced in these 11 week post TDA-fed animals.

Significance

These studies demonstrate that TDA exerts a toxic effect on spermatogenesis in the male rat. The results indicate that TDA is capable of reducing fertility and of exerting an inhibitory effect on sperm production at nontoxic doses. It appears that TDA has deleterious effects on androgen production and action, but may not be capable of inducing mutagenic changes in the germinal cell of the male rat. The diminished cauda epididymal sperm counts 11 weeks after TDA treatment, suggests that the agent induced damage to the spermatogonical stem cells. The precise mechanism by which TDA exerts its adverse reproductive effects remains to be established.

Publications - None to date

REPRODUCTIVE EFFECTS OF LEADS OR SOLVENT EXPOSURE AT WORK

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Reproductive Disorders
5 R01 OH01910-02
04/01/84 - 03/31/87
$66,572 ($113,267 Cum)

Objectives

The aim of this study is to investigate, whether exposure of men and women to organic solvents or inorganic lead at work causes spontaneous abortions or malformations in the offspring.

Methodology

The exposed group consist of women and men who, according to the biological monitoring data, have been exposed to organic solvents or lead through 1972 to 1982. The cases of spontaneous abortions will be identified from the hospital discharge register maintained at the National Board of Health. A possible high risk for congenital malformations in the children of the exposed women can be excluded or confirmed by using the Finnish Register of Congenital Malformations. The figures will be compared with controls who have born a normal child.

Progress & Accomplishments

The collection of the biological measurements of solvent exposure as a computerized data file with the personal identification codes has been completed. The most common solvents monitored were trichloroethylene and styrene.

The pregnancies of the monitored workers have been identified from the Hospital Discharge Register and the Finnish Register of Congenital Malformations. The women had 1440 pregnancies from 1973 to 1983 and the proportion of spontaneous abortions to all pregnancies was 9.7%. The wives of monitored men had 4035 pregnancies and the proportion of spontaneous abortions among them was 8.8%. The number of children with congenital malformation was 14 among monitored women and 39 among the wives of monitored men.

The investigation was continued as a case-control study. All women who had been treated at hospital for spontaneous abortion or who had a malformed child were defined as cases. Three age-matched controls were selected for spontaneous abortion-cases and five controls for malformed children from the women who had given birth to a normal child. Similarly, cases and controls were selected from the wives of solvent exposed men. A questionnaire has been sent to the women or to the men and their wives to confirm the solvent exposure during the first trimester of pregnancy.

The collection of biological monitoring data of lead exposed workers has been completed. The number of biological measurements was about 60,000. The checking and correction of the data file is in progress.

Significance

The anticipated result: the possible effects of organic solvents or lead at various exposure levels on the pregnancy outcome of the workers are in prevention of health hazards. They can also be helpful for the understanding of dose-effect relationships and for the planning of occupational hygienic standards.

Publications - None to date

EFFECTS OF 27MHZ RADIATION ON SOMATIC AND GERM CELLS

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Reproductive Disorders
1 R01 OH02148-01
09/27/85 - 08/31/88
$108,768 ($108,768 Cum)

Objectives

To determine thresholds and dose-response relationships for the effects of 27-MHz radiofrequency radiation (RF) on mammalian somatic and germ cells *in vitro* under accurately measured and precisely controlled exposure conditions. The extent to which effects are dependent upon RF frequencies, specifically 1-, 10-,50-,100-MHz, and 2.45-GHz.

Methodology

Well-characterized somatic cells (erythrocytes, neutrophils, glioma [C5 and LN-71], fibroblasts, and HeLa) and germ cells (mouse ova and sperm) will be exposed under conditions of accurately measured electric and magnetic field strength in a temperature-controlled coaxial line RF cell exposure system or a temperature-controlled microwave waveguide exposure system. The dependent variables will include: 1) cell viability and morphology, 2) phagocytosis and chemotaxis, 3) plasmalemma cation permeability, 4) cell cycle regulation, 5) DNA and protein synthesis, 6) sperm viability and motility, and 7) fertilization (*in vitro*).

Progress & Accomplishments

Frequency dependence of cellular responses *in vitro* has been investigated by simultaneous exposure of cell cultures to 27-MHz or 2450-MHz CW radiation at identical SAR's under precise temperature control (37 ± 0.2'C). Exposure of human glioblastoma cells (LN-71) at 80 W/kg for 2 h to 2450-MHz microwaves resulted in metabolic stimulation as indicated by increased uptake of labeled thymidine and uridine and mitochondrial activity at 3 and 5 days post exposure. Simultaneous exposure of the same cell line to 27-MHz RF radiation, at the same SAR, resulted in an apparent metobolic suppression, as indicated by decreased uptake of thymidine and uridine on days 3 and 5 post exposure, with no effect on mitochondrial activity. Exposure to either frequency did not cause morphological alterations as assayed by phase contrast light microscopy. Preliminary studies of the comparative effects of these frequencies (also at a SAR on 80W/kg) on structure and function of mouse sperm have been undertaken.

Significance

Data generated by this study will be used in the assessment of potential health risks associated with occupational exposure to 27-MHz RF radiation generated by RF heat sealers, diathermy equipment and other devises that are known sources of human exposure. Since such devices are generally operated intermittently, comparative studies will be conducted using continuous wave and amplitude modulated 27-MHz RF.

Publications - None to date

ENVIRONMENTAL/INDUSTRIAL TOXICANTS AND TESTICULAR INJURY

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Reproductive Disorders
1 R01 OH02191-01
09/27/85 - 08/31/88
$113,316 ($113,316 Cum)

Objectives

Studies in our laboratory have resulted in a model of γ-diketone intoxication which permits dissection of biochemical and morphological events specific for testicular atrophy. In preliminary experiments, we have demonstrated alterations in microtubule assembly which occur in association with testicular atrophy induced by the γ-diketone 2,5-hexanedione. The alterations consist of earlier and more rapid microtubule assembly. This assembly behavior is explained by the presence of additional microtubule nucleating centres. We have tentatively identified as a potential nucleating center a covalently cross linked tubulin dimer present in pure tubulin isolated from intoxicated rats. Based on these results regarding the role of tubulin in γ-diketone induced testicular atrophy, we propose the following working hypothesis: 2,5-hexanedione reacts with tubulin lysyl residues to form pyrroles which form covalent cross links between tubulin monomers; covalently cross linked tubulin multimers change the *in vitro* and *in vivo* kinetics of microtubule assembly producing shorter and more numerous microtubules; microtubule modification is manifested as testicular injury because of the unique dependence of the testis on the cytoskeletal integrity of the Sertoli cell.

The goal of this project is to test this hypothesis of the pathogenesis of toxicant induced testicular atrophy by fulfillment of the following objectives:

1. To utilize a γ-diketone treatment protocol to specifically correlate the temporal onset and development of testicular microtubule alterations with the appearance of testicular atrophy.
2. To evaluate the reversibility of 2,5-hexanedione induced testicular atrophy in conjunction with biochemical resolution of the microtubule abnormalities.
3. To investigate the consequences for cells and tissues, particularly Sertoli cells and germ cells in the testis, of microtubule dysfunction caused by intoxication.
4. To delineate the chemistry and biochemistry of the tubulin modification which results in abnormal microtubule assembly kinetics.
5. To determine the generality of the hypothesis by testing microtubule properties in testicular atrophy induced by additional γ-diketones, the acrylamides and carbon disulfide.

Methodology

First priority will be given to establishing an efficient and reproducible method for isolating assembly competent pure tubulin from rat testis. The assembly kinetics of pure rat testis tubulin from control, 2,5-hexanedione and 3,4-dimethyl-2,5-hexanedione treated animals will then be compared, along with additional biochemical and morphological parameters, during a time sequence study of the induction and resolution of the testicular atrophy. Separation of Sertoli cells from germ cells combined with electron microscopy and anti-tubulin antibody techniques will be used to focus attention on the specific cell type within the testis which is injured by 2,5-hexanedione. Tubulin cross linking will be explored as the molecular mechanism for 2,5-hexanedione induced changes in microtubule assembly. Finally, other compounds will be evaluated for inclusion within the class of testicular toxicants which act via microtubule modification.

Progress & Accomplishments

This is a new project.

Significance

The proposed studies will further the understanding of the basic mechanisms underlying toxicant induced testicular injury. The alterations in microtubule assembly kinetics induced by 2,5-hexanedione are seen as a manifestation of the cytoskeletal disruption which results in testicular atrophy. A combination of morphological and biochemical techniques will be utilized in localizing the microtubule alteration to a particular cell type within the testis and defining the chemical nature of the tubulin modification. The significance of this proposal lies in the ability to generalize from the proposed subcellular mechanism to include those agents which act in a similar fashion. Also, during the course of the project, methods will be developed which readily allow early assessment of new agents which may be testicular toxicants.

Publications - None to date

ROLE OF GAP JUNCTIONS IN ADVERSE REPRODUCTIVE OUTCOME

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Reproductive Disorders
5 K01 OH00024-02
09/28/84 - 08/31/87
$31,536 ($63,126 Cum)

Objectives

It is hypothesized that alteration of junctional communication may be one mechanism underlying adverse reproductive outcome and that this phenomenon may serve as the basis for rapid screening assays. The specific objectives of the research are to:

1. Test known and potential human reproductive toxicants for inhibition of metabolic cooperation, one manifestation of junctional communication, among cells in culture.
2. Test whether substances which block metabolic cooperation in cultured cells also interfere with hydra reaggreation.
3. Evaluate gap junction morphology in hydra reaggregates exposed to inhibitors of junctional communication.
4. Evaluate dye-coupling in reaggregating hydra at various stages of morphogenesis.
5. Test known and suspected teratogens for their ability to alter the morphogenetic process of hydra reaggregation, and correlate this with effects on dye-coupling in the hydra reaggregates.

Methodology

Inhibition of metabolic cooperation is monitored in cultured cells using the V79 cell system of coculturing wild-type, 6-thioguanine (6-TG) sensitive and mutant 6-TG resistant cells in the presence of G-TG and the test compounds. Inhibition of metabolic cooperation is indicated by the increased survival of the mutant cells due to blockage of intercellular transfer of the toxic 6-TG metabolite. We have also begun experimentation using "scape-loading" as a method of incorporate fluorescent dyes into cultured cells for dye-coupling experiments. The dye becomes incorporated into cells when the cells are scraped from the surface in media containing the dye. Once inside the cell, the hydrophilic dye does not readily cross the plasma membrane, and its spread to contiguous is presumably through communicating membrane junctions.

To examine the effects of inhibition of junctional communication on a developing organism, we use the hydra reaggregation model as a test system for morphogenesis studies. Hydra cells will reform the adult structure after dissociation into single cells if the cells are brought into close enough proximity. The reaggregation process includes many of the phenomena exhibited by mammalian embryogenesis.

Progress & Accomplishments

1. Cell culture experiments. In the first year of the project we have completed an evaluation of a series of toxic metals and their ability to inhibit junctional communication in the V79 cell coculture system. We demonstrated that several of the toxic metals inhibited metabolic cooperation at noncytotoxic concentrations, including arsenic acid, mercuric chloride,

and lead acetate. Cadmium acetate inhibited metabolic cooperation only at cytotoxic metabolic cooperation at any concentration. These data will be presented at the 1986 Meeting of the Society of Toxicology, and are being prepared for publication. We have begun examining whether these effects are duplicated in ameliorate the inhibitory effects of the other metals.

We have also tested whether the putative toxic metabolites of the reproductive toxicants, 2-methoxyethanol and 2-ethoxyethanol, could mimic the parent compounds' ability to inhibit metabolic cooperation in V79 cells. We demonstrated that the metabolites, 2-methoxyacetic acid and 2-ethoxyacetic acid, were much more cytotoxic to the cells than the parent compounds, but that they did not inhibit metabolic cooperation. These data have been submitted for presentation at the 1986 Meeting of The Tissue Culuture Association.

2. Hydra reaggregation experiments. We have tested a series of phorbol compounds for their ability to interrupt hydra reaggregation. The series includes the potent inhibitor of junctional communication, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), and intermediate-strength analogues. We found an approximate correlation between the potency of the phorbols to inhibit metabolic cooperation in cultured cells and interrupt hydra reaggregation. However, the phorbols did not show a preferential toxicity to the reaggregates, since toxicity curves for the adult hydra were similar to those of the reaggregates. Because data in the literature suggest that junctional communication is important in the morphogenesis of a variety of species, we hypothesize that it is also important for maintenance of the adult hydra. We have prepared specimens of adult and reaggregating hydra exposed to TPA or solvent (controls) for electron microscopic examination. We will study whether the gap junction morphology is altered in response to the phorbol treatment and whether this occurs in adults and reaggregates at similar concentrations. This will afford up some insight into the possibility that junctional communication may be of equal importance to the adult and reaggregating forms. It is also possible that the phorbol compounds affect the V79 cultured cells and hydra differently, since some cell-specificity has been noted in the literature. We will pursue this possibility by testing several other substances known to inhibit junctional communication in other systems. Specific binding of the membrane in a receptor fashion has been observed in hydra, however, suggesting that hydra respond in at least this fashion.

Significance

The experiments performed in the first year of the grant have advanced the project closer towards the goals of understanding the role of junctional communication in reproduction and development, and developing rapid screening systems based on this phenomenon. We have demonstrated that some known reproductive toxicants inhibited metabolic cooperation in the cell culture system. The studies with the hydra have advanced the evaluation of hydra reaggregation as an appropriate model for morphogenesis studies. Our future directions will expand the cell culture studies to other cell systems, including human cells and metabolism-competent systems, towards the goal of developing a culture system that is an analogous to the human situation as possible. Future hydra experiments will continue evaluation of the hydra reaggregation model. If the hydra reaggregation model proves to be useful for the evaluation of the role of junctional communication in developmental phenomena, it will provide a rapid and easily manipulated whole-animal system for future research.

Publications - None to date

GENOTOXIC EXPOSURE ASSESSMENT BY SIMPLIFIED DNA ANALYSIS

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Reproductive Disorders
5 K01 OH00035-02
09/28/84 - 08/31/87
$32,223 ($64,375 Cum)

Objectives

The objective of this project is to use available molecular biological methods to develop a sensitive and practical test for *in vivo* human exposures to genotoxic agents. The test is to be based on readily obtained specimens, such as peripheral blood leukocytes.

Methodology

The primary material used in this procedure is DNA extracted from Leukocytes separated from freshly drawn human blood. (Ultimately, more refined separation techniques will be used to separate T-lymphocytes.) Aliquots of the freshly drawn blood are subjected to treatments (ionizing radiation, chemical mutagens, or control treatment) while held at 37 degrees. The 340 base pair alphoid sequence is separated from the DNA by Eco R-1 restriction, then labeled and subjected to secondary chemical or enzymatic treatment to produce breakage at damaged sites. Breakage is detected by subjecting the treated material to electrophoretic or column chromatographic treatment, followed by autoradiography or liquid scintillation counting, as appropriate.

Progress & Accomplishments

The primary areas of activity during the first year were investigations of the normal variability of the 340 BP DNA within an individual and the nature of alterations produced in the 340 BP sequence upon treatment with chemical agents, and the development of a highly radioactive 340 bp probe. Much of this work was performed on stocks and clones of human placental DNA on hand at the beginning of the project. Dimethyl sulfate (DMS) was used as a chemical genotoxin, and gamma radiations from cobalt-60 and cesium-137 were used as physical agents. The indicator sequence was exposed to DMS under various conditions and a distinct and reproducible pattern of abberations was shown to occur. Data from this procedure will be used to develop shorter and more sensitive techniques suitable for routine analysis of field samples. Preliminary steps have been taken to bind radioactively labeled 340 BP fragment DNA with restricted DNA sections that have been transferred to nitrocellulose. Progress is being made to fully develop this shorter technique so that it can be applied in the studies of DNA response to ionizing radiation and chemical mutagens.

Significance

The development of a practical assay for genetic damage in the occupational setting would have a number of practical implications, the most immediate being a supplement to conventional personnel monitoring. While an assay for general genetic damage will almost certainly lack agent specificity, the potentially genotoxic agent is known in many routine occupational situations and in many cases of accidental exposures. Another application would be the assessment of genetic damage in exposure condition so complex that traditional assessment methods cannot produce a meaningful interpretation of the situation. As data on various groups accumulates, the possibility exists that tests developed in this project would be of some value as predictors of genotoxin-induced disease.

Publications - None to date

ALCOHOL ALTERATIONS OF PRENATAL WORKSHOP CARCINOGENESIS

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Reproductive Disorders
5 R03 OH01705-02
07/01/83 - 06/30/85
$18,644 ($34,421 Cum)

Objectives

The goal of this project was to apply our *in vivo* bioassay for delineating the effects of *in utero* exposure to alcohol and workplace carcinogens in immediate and future offsprings.

Methodology

Testing of this hypothesis was carried out by measuring the specific antitumor cell-mediated immunity (CMI) in Fischer F344 inbred rats brought about by exposure to 1,2-dimethylhydrazine (DMH) and alcohol. Pregnant rats were administered a 5% ethanol solution on day 13 of gestation. On the 16 to 18th day of gestation each dam was subcutaniously injected with varying doses of DMH. To avoid the problem of lack of "pair-feeding" between animals exposed to alcohol and nonexposed controls, both groups of animals were maintained on a caloric balanced "Iso-Cal" diet. Using a radiometric release CMI assay, antitumor measurements were determined 60 days post-partum for the dams and the F_1, F_2, and F_3 offspring.

Progress & Accomplishments

The specific goals proposed in this grant were accomplished and the results described as follows. Initial studies involved the exposure of pregnant rats to a 5% solution of ethanol beginning at the 6th day of pregnancy. On the 16th-18th day of gestation, alcohol consumption was terminated and the dams injected with DMH. However, due to the high spontaneous abortion rate (greater than 85%) using this protocol, the length of time each dam was exposed to alcohol was shortened allowing for a higher birth rate.

An examination of the average litter size of the test families suggests that alcohol may be fetal toxic. Dams exposed to alcohol and either saline or DMH had fewer pups than dams exposed to only saline or DMH. A shift in the sex ratio from a higher incidence of males in families exposed to only DMH to a greater number of females in families exposed to alcohol was also detected.

The majority of pups exposed to alcohol and DMH responded with CMI activity, however, the response was quite variable between family members and families. Pups exposed to alcohol only also developed a CMI response of comparable intensity. This was unexpected since alcohol was considered to be noncarcinogenic. Dams evaluated during this same time period demonstrated a CMI response as well. This was in contrast to previous work in which a CMI response was not detected in dams exposed to only DMH. Additional experiments on adult animals exposed to alcohol and DMH suggests that alcohol enhances CMI responsiveness to DMH in males while it diminished in females.

Significance

The present findings support the proposed hypothesis that alcohol consumption will modify the ability of carcinogens to induce cancer. This contention wes demonstrated by the effect alcohol had on DMH's ability to induce an antitumor immune response (CMI response) in adult animals and in offspring exposed *in utero*. It was also shown that the animals exposed *in utero* to alcohol and DMH were more sensitive than adult exposed animals. If this is true, such an increase in sensitivity should be considered when regulations are set for workplace carcinogens.

Publications - None to date

EFFECT OF BENZENE ON FETAL HEMATOPOIESIS

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Reproductive Disorders
5 R03 OH01713-02
07/01/83 - 06/30/85
$17,980 ($36,951 Cum)

Objectives

The purpose of this study is to evaluate the effects of low level maternal inhalation exposure to benzene on fetal, neonatal, and young adult erythrocytic hematopoeitic and granulocytic cell lines. In this way, the consequence of low concentrations of benzene on the developing hematopoietic system can be studied under realistic exposure conditions.

Methodology

Experiment 1 - The hematotoxic responses to in utero exposure to 0, 5, 10 or 20 ppm benzene were determined in fetuses on the 16th day of gestation. At this time, the number of live, dead, and resorbed fetuses were recorded. Two male and two female fetuses were then randomly selected, weighed, and examined for any external gross morphological malformation. Peripheral blood samples were taken for red and white cell counts and for hemoglobin analysis. Livers were then removed aseptically for committed stem cell assays (BFU-E, CFU-E, CFU-GM).

Experiment 2 - The hematotoxic responses to in utero exposure to 0, 5, 10, and 20 ppm benzene were determined in two day old neonates. The exposed pregnant females were allowed to proceed through normal parturition. Two male and two female neonates were then randomly selected at 2 days of age and subjected to the same protocol as that described above with 16-day old fetuses.

Experiment 3 - The persistence of any hematopoietic disruptions due to in utero exposure to 0, 5, 10, and 20 ppm benzene was investigated in 6-week old adults. Exposed pregnant dams were again allowed to proceed through normal parturition. The offspring were monitored daily for morbidity and mortality. Neonatal weights were taken on the second day after birth and once a week thereafter. At six weeks of age, one male and one female were randomly selected from each litter. Peripheral blood samples were obtained from tail veins for red and white cell counts and for hemoglobin analysis. Hematopoietic progenitor cell assays were performed on splenic and marrow cells from these animals.

Experiment 4 - The long terms effects of in utero exposure to 10 ppm benzene was investigated in ten week old progeny. These animals were from the same litters as those used in experiment 3. Ten week old animals which were exposed in utero to 10 ppm benzene were split into two groups of 5 males and 5 females each. These two groups were then re-exposed for 2 weeks to either filtered air or 10 ppm benzene. Animals exposed in utero to air were also split into two groups and re-exposed to either air or benzene. After the 2 week exposure period the animals were subjected to the same protocol as that described for the 6 week old adults.

Progress & Accomplishments

There was no evidence of conventional maternal toxicity at any concentration of benzene employed. There was also no evidence of conventional toxicity in any of the progeny of the exposed dams except for reduced rates of weight gain, from birth to 6 weeks, for those progeny from dams exposed to 10 ppm or 20 ppm benzene. In addition, litters exposed in utero to 20 ppm benzene displayed elevated incidences of mortality.

All concentrations of benzene employed induced marked changes in several hematopoietic parameters among progeny of exposed dams. Particularly striking were the changes induced to erythroid populations although granulocytic cell populations were also affected by the exposures. Fetuses exposed in utero to 5 ppm or 10 ppm benzene displayed elevated BFU-E and CFU-E colony growth and elevated peripheral red blood cell counts. In contrast, those fetuses exposed in utero to 20 ppm generally displayed reductions in these parameters. Neonates exposed in utero to all three benzene concentrations displayed altered CFU-E colony formation but even more dramatic were the effects observed on CFU-GM colony formation. No changes in this parameter had been observed among in utero exposed fetuses but neonatal progeny exhibited marked changes. Those exposed to 5 ppm and 10 ppm benzene exhibited reduced CFU-GM colony formation while those exposed to 20 ppm exhibited elevated colony formation. Some long-term lingering effects of the in utero exposures were also observed as 6-week old adult progeny of the exposed dams displayed elevated peripheral red blood cell counts. Interestingly, all concentrations of benzene studied elicited this response. Of greater interest, when 10-week old adult mice which had previously been exposed in utero to 10 ppm benzene were re-exposed to 10 ppm benzene for 2 weeks they displayed markedly reduced CFU-E colony formation. Apparently, the ability of these mice to withstand benzene exposure as adults had been compromised by their in utero exposures.

Significance

This study demonstrates marked effects on the hematopoietic system of fetal mice exposed in utero to 5, 10, and 20 ppm benzene. Ten ppm is currently considered safe for occupational exposures. The most dramatic effects were seen in the fetal and neonatal erythron, with extreme changes in colony growth of both the BFU-E and CFU-E precursor cell populations. These effects were reflected by commensurate changes in the peripheral erythrocyte counts. Significant effects on the granulocytic system were also observed in 2-day old neonates. We also observed long-term effects of in utero benzene hematotoxicity as evidenced by a reduction in neonatal weight gain and by increased sensitivity to the hematopoietic stress of low-level, short-term re-exposure to benzene as adults.

With regard to the possible extrapolation of these findings to the risk of human in utero exposure, it should be noted that the human fetus possesses a greater ability to metabolize xenobiotics in comparison to the mouse fetus. Since metabolites of benzene are believed to be the agents ultimately responsible for its toxicity, a human fetus may be exposed to more toxic benzene metabolites than a mouse fetus undergoing comparable maternal exposure.

Publications - None to date

OCCUPATIONAL FACTORS ASSOCIATED WITH BILIARY ATRESIA

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Reproductive Disorders
5 R03 OH01827-02
09/28/84 - 08/31/86
$5,080 ($28,995 Cum)

Objectives

Empirical observation has raised suspicion that parental occupational factors may be associated with Congenital Biliary Atresia ("B.A.") in the offspring. The principal objective of this exploratory study is to determine whether parentally reported occupations, types of employment, and/or selected chemical exposures related to the pre-conceptual, gestational, and/or neonatal periods constitute significant factor(s) associated with the occurrence of B.A., or a subgroup thereof.

Additional aims of this study include determination of:

1. Associated, selected home or avocational exposures
2. Whether teratogenic associations are related to paternal or maternal exposures, or to specific combined exposures (interactions)
3. Increased incidence of other teratogenic outcomes (including spontaneous abortion) in case sibships (correlated with parental occupation for each pregnancy)
4. Associated familial occurrence of hepatitis (both Type B and others), and other specific conditions
5. Maternal health status and perinatal complications
6. Selected demographic and socioeconomic variables

Methodology

This case-control study has selected a consecutive series of Extra-hepatic B.A. cases born within the four-year span from 1982 through 1985, from the National B.A. Registry of the American Academy of Pediatrics. For improved ascertainment, all Institutions referring the the Registry are being asked to review their files and to also include the newer (infant) cases as well as any earlier cases not yet registered. Desired sample size for cases is 300 to 400 or more.

Controls, matched with respective cases on sex and age (D.O.B. within one year), and on race whenever feasible, are being collaboratively selected through the files of each respective case surgeon. They will have had surgery under twelve months of age, for one of several non-congenital abdominal conditions (including inguinal hernia, intussusception, others). A second, population-based ("Normal") comparison group will soon be available per the data from the contemporary retrospective study on Congenital Heart Disease through the courtesy of Dr. Charlotte Ferenz, M.D., M.P.H. of the University of Maryland, School of Medicine. Validation of case and control diagnoses, and obtainment of selected clinical data, will come through receipt and review of selected medical histories. Parental data will be obtained through mailed, self-administered written questionnaires, separate ones for mothers and for fathers. Each questionnaire covers relevant job history, selected exposure history, hobby and other environmental history, reproductive and extended family history, and parent's personal health history.

Follow-up with non-responder parents will entail initially sending a "reminder" post-card and then, if no subsequent response, direct phone contact(s). For those families with apparent communication or literacy problems, we will offer telephone interviews. Data collected by different modes will be stratified for comparisons.

Distribution and cross-tabulations of various occupations, occupational groupings (based on similar chemical exposures), and other environmental factors will be done by case-control status, after adjustment for potential environmental, socioeconomic, and prenatal and perinatal confounders. Multiple regression analysis will be used to weight significant variables, identify significant interactions, and adjust for confounding variables. Maternal and paternal data will be analyzed separately as well as jointly, for suspected interaction effects.

Progress & Accomplishments

1. Ongoing, close collaboration with the B.A. Registry has been maintained.

2. In the Fall of 1985, correspondence (under the joint signatures of Dr. John R. Lilly, Director of the Registry, and of this P.I.) to all the Registry referral institutions/surgeons informing them of the up-coming study and requesting their collaboration. Many have already responded positively.

3. The Pre-testing Phase is presently underway and will be concluded in February. A sample of parents of Baltimore and Maryland based children (born between 1982 and 1984) with abdominal congenital anomalies has been employed and data forms are being assessed for effectiveness and clarity.

4. Direct, ongoing long-distance collaboration with the participating medical institutions will commence on 1-30-86 and continue over several months until all demographic file data is obtained.

5. Data collection will begin in March and run through the summer months, as necessary, and will include the following activities: Mailing out of the parental questionnaires, following up with "non-responder" families, requesting of medical histories from the institutions, and coding of data as it is received.

Significance

Not yet applicable.

Publications - None to date

TOXICOLOGY OF INDUSTRIAL CHEMICALS ACTING ON THE CNS

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Neurotoxic Disorders
5 R01 OH00535-09
04/02/82 - 03/31/85
$111,742 ($807,725 Cum)

Objectives

These studies were designed to meet some of the research needs outlined in National Institute for Occupational Safety and Health Criteria Documents for Acrylamide (1976) and Alkanes (1977) and elsewhere in U.S. Environmental Protection Agency reports on Acrylamide (1976, 1977) and Diketone Solvents (1977); to develop and utilize new, sensitive methods for the assessment of chemically-induced neurotoxicity; and to illuminate the mechanisms of neurotoxin-induced nerve-fiber degeneration.

Methodology

Experimental studies in laboratory animals utilized light and electron microscope techniques to examine the effects of acute and chronic exposure of neurotoxic industrial and other chemicals on the brain, spinal cord and peripheral nerves of developing, adult and aged animals.

For clinical studies, the short latency somatosensory evoked potential (SEP) procedures developed in the initial year of this project were used to detect the earliest signs of neurotoxic injury. Two additional novel techniques have been utilized, one for vibratory/tactile assessment (using the modified Optacon), and the other for thermal sensation.

Progress and Accomplishments

Experimental studies examined the neurotoxicity of the vinyl monomer acrylamide and the ketone solvent 2,5-hexanedione (the ultimate toxic metabolite of the hexacarbon solvents *n*-hexane and methyl *n*-butyl ketone) and certain other compounds of industrial/environmental significance. Light and electron microscope examination of central and peripheral nervous system tissues taken from animals exposed to these agents during different stages of development demonstrated a specific pattern of central-peripheral distal axonopathy. These represented the first detailed morphological studies of experimental occupational toxic neuropathy, studies which heavily influenced understanding of the mechanisms underlying these diseases. These prompted a number of biochemical studies which examined the action of acrylamide and 2,5-hexanedione on selected enzymes in intermediary metabolism. Results of these studies led to the development and testing of a hypothesis on the mechanisms underlying neuropathy induced by a number of chemically unrelated substances.

Human subjects evaluated with the SEP procedures included individuals intoxicated with such substances as acrylamide, pyridoxine, organic solvents, pesticides, and mercury. In addition, patients with a variety of confirmed or suspected neurological disorders were evaluated and compared with subjects exposed to neurotoxins. For each of these groups, the short latency SEP components provided a valuable index of the onset and severity of conduction slowing in distal portions of CNS axons. The total central transmission time proved the most consistent and sensitive measure of neuropathy. In many patients, distal CNS dysfunction was evident prior to manifestations of change in the more proximal portions of the central axons or in the PNS neurons.

Our experience with the SEP also illustrated the limitation of this procedure for the routine assessment of neurotoxicity. Data collection is time-consuming and relatively expensive, and data interpretation requires a high degree of technical sophistication.

A major accomplishment was the development of instrumentation and procedures for the quantitative assessment of sensory thresholds. These measures are rapid, reliable, non-invasive and sensitive to dysfunction within large and small neurons. The testing methods selected utilize a "two alternative forced choice" procedure, interfaced with a computer generated algorithm. Normal population data have been obtained for more than 500 subjects addressing age effects, variability estimates and correlations with standard electrophysiological and clinical tools. Clinical studies originating from this work include investigations of subjects exposed to Taxol, pyridoxine, lead, methyl mercury, dioxin, and solvents, as well as subjects with diabetes, kidney failure and surgical nerve repair.

Investigations supported by this grant also contributed to and edited a major textbook on experimental and clinical neurotoxicology.

Significance

Experimental animal studies have advanced our understanding of the site of action of occupational neurotoxins and have shown the distal parts of central and peripheral nerve fibers to be the most vulnerable to systemic intoxication. Novel hypothesis have been developed to account for the mechanisms underlying nerve fiber degeneration induced by these agents.

Clinical studies have demonstrated a useful method for monitoring "at risk" populations for early signs of neurotoxic injury.

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OCCUPATIONAL NEUROPATHIES DUE TO INDUSTRIAL CHEMICALS

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Neurotoxic Disorders
5 R01 OH00823-06
08/01/82 - 07/31/85
$124,851 ($549,061 Cum)

Objectives

Many chemicals produce neurotoxicity in humans and some animal species. Toxicological studies and epidemiological evidence have implicated simultaneous exposure to the organophosphorus insecticide leptophos and the industrial solvents, *n*-hexane and toluene, caused an outbreak of neuropathy among workers handling these chemicals. Leptophos belongs to a group of chemicals capable of causing organophosphorus compound-induced delayed neurotoxicity (OPIDN). The adult hen is the animal of choice to study OPIDN since rodents are not as sensitive. *n*-Hexane and Toluene has been implicated in causing neurologic deficits in humans. Because humans are often exposed concurrently to chemicals from these groups, the overall objective of this project is to study the joint neurotoxic action of these neurotoxicants.

Methodology

The adult hen was used as the test animal since it is sensitive to neurotoxicity induced by organophosphorus and aliphatic hexacarbon-type chemicals. Dermal and inhalation exposure were employed since they are the major routes of occupational exposure, to these chemicals both by industrial and agricultural workers. The organophosphorus chemical used was the delayed neurotoxic insecticide EPN (*O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate) and the neurotoxic aliphotic hexacarbons used were: *n*-hexane, 2-hexanone (M*n*BK), 2,5-hexanediol (2,5-HDOH), and 2,5-hexanedione (2,5-HD). The non-neurotoxic methyl *iso*-butylketone (MiBK), which has replaced the neurotoxic M*n*BK in industry, was also used in some experiments.

Progress & Accomplishments

In the first phase of these studies, the hen was established as a model test animal for aliphatic hydrocarbons via intraperitoneal injection, inhalation, and oral and dermal administration. The relative neurotoxic effect of aliphatic hexacarbons in hens in descending order was: 2,5-HD 2,5-HDOH M*n*BK *n*-hexane. The results showed that although both aliphatic hexacarbons and organophosphorus compounds are capable of producing similar signs of neurotoxicity in the hen, the morphology and distribution of the neuropathologic lesion were distinct. Concurrent inhalation of methyl butyl ketone (MBK) and dermal application of EPN potentiated the neurotoxic effect of either chemical above. Also, the non-neurotoxic methyl *iso*-butyl ketone (MiBK) synergized the neurotoxic effect of the weak neurotoxicant *n*-hexane in hens. Simultaneous dermal exposure to EPN and *n*-hexane and its metabolites produced either additive or potentiating effect. The neurotoxic chemicals M*n*BK, MiBK, and EPN induce chicken hepatic microsomal cytochrome P-450. Also, dermal application of *n*-hexane, M*n*BK, and 2,5-HD enhanced the dermal absorption of EPN.

Significance

The results of this project have demonstrated that in the test animal (the hen): a) simultaneous dermal administration of EPN and inhalation of MBK vapor potentiated their neurotoxic effects, b) persistent dermal exposure to small daily doses, without skin protection, of n-hexane, MnBK, 2,5-HDOH, or 2,5-HD in combination with EPN produced a potentiating or additive action on EPN neurotoxicity in the following order: 2,5-HD MnBK 2,5-HDOH n-hexane, and c) concurrent exposure to n-hexane and MiBK vapors greatly enhanced the neurotoxicity of n-hexane alone. The mechanisms of these joint actions are being investigated in the current phase of the project. Since humans are also susceptible to neurotoxicity produced by these chemicals, these results emphasize the need to prevent skin contact in industrial and agricultural workers required to handle these types of chemicals. It is also prudent that both management and workers be aware of the hazards resulting from washing skin contaminated with organophosphorus compounds using aliphatic hexacarbon solvents.

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MECHANISMS OF TOXIC OCCUPATIONAL NEUROPATHY

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5 R01 OH00851-06
05/01/83 - 04/30/86
$176,554 ($912,712 Cum)

Objectives

These studies seek to illuminate the role of axon transport in the induction of nerve-fiber degeneration induced by agents of industrial significance.

Methodology

Axonal transport studies utilize radiolabeled markers to determine the rate of anterograde and retrograde transport and the amount of material transported in intact and injured nerves of intoxicated animals. Biochemical assay procedures are carried out to examine enzyme activity in treated nerves and to study the relationship between enzyme inhibition and nerve-fiber degeneration. Light and electron microscope techniques are used to examine central and peripheral nervous system tissues taken from intoxicated animals. In addition, a novel organotypic tissue culture system consisting of fetal mouse spinal cord, dorsal root ganglia and muscle is utilized to monitor the effect of neurotoxic compounds on living nervous system tissues during intoxication and recovery.

Progress & Accomplishments

The occupational neurotoxin acrylamide, induces distal, retrograde degeneration of peripheral nerves in intoxicated rodents. Our studies have shown that acrylamide-induced axon degeneration is preceded by dose-dependent changes in retrograde axonal transport that are temporally related to deleterious alterations in the neuron perikaryal repair responses to injury. This has led to the suggestion that acrylamide, and perhaps other agents that precipitate toxic axonal neurophathies, impede information transfer from axon to perikaryon that is required to activate repair responses needed for the maintenance of axonal integrity.

We have also examined, for the first time, the entire sequence of pathological changes in hexacarbon neuropathy using 2,5-hexanedione (the ultimate metabolite of n-hexane) and the more potent compound, 3,4-dimethyl-2,5-hexanedione (DMHD), in living organotypic tissue cultures. Our findings demonstrate different pathological responses in cultures as a consequence of dose and potency. In the presence of DMHD, neurofilament accumulation (which causes axonal swellings) and axonal degeneration appear to be two separate pathological events, both spatially and temporally. Therefore, it is unlikely that chemical interference of neurofilament transport is the sole cause of hexacarbon-induced axonal degeneration.

These studies have been supplemented by an analysis of the toxic neurological disorders induced by other substances (e.g., 2-t-butylaxo-2-hydroxy-5-methyl hexane) of industrial/environmental significance.

Significance

Peripheral neuropathy associated with alterations in axonal transport has resulted from human overexposure to a number of industrial chemicals, including the compounds studied here (i.e., acrylamide and *n*-hexane derivatives). The molecular and cellular mechanisms underlying the subchronic neurotoxicity of these agents is not well understood and under intensive study. We have made a major advance by defining the pathophysiological changes in nerve fibers which percede degeneration induced by certain chemical agents.

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CHRONOBIOLOGY AND OCCUPATIONAL HEALTH HAZARDS

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2 R01 OH00952-07
12/01/84 - 11/30/87
$114,594 ($726,670 Cum)

Summary

The long-term objective of this study has been to determine what role metabolic rhythms have on influencing response to toxicity from physical stimuli, industrial toxins and therapeutic agents. Among those that have been, or currently are being investigated include: irradiation, paraquat, urethane, malathion, mercuric chloride, insurin, glucagon, epidermal growth factor (EGF), somatostatin, ACTH, and gastrin. The toxicity response in mice to all has been shown to be circasian-stage dependent. Moreover, some of the above polypeptides stimulate RNA and DNA synthesis in a number of tissues including the alimentary canal (EGF, insulin and gastrin) whereas others are predominantely inhibitory (glucagon, somastatin, and ACTH), again all responses have been found to be circadian-stage dependent. The aim of the present application is to continue such studies, but also, to determine if the chronobiological findings already gained can be used to reduce overall toxicity.

The model chosen has been cell proliferation in certain regions of the intestinal tract, which also undergoes remarkable circadian variation. Can the fact that fasting, beginning at a certain circadian stage, for a span of 36 hours or less reduce the fraction of proliferating cells and thus be used to protect the gut from damage brought about by: (1) a physical agent such as irradiation which affects the mitotic spindle or (2) a chemical agent such as cytosine arabinoside, which specifically interfers with DNA synthesis? Moreover, will any of the above mentioned peptides, when given before, simultaneously, or subsequent to either a physical or chemical induced injury, protect different regions of the gut from damage or enhance repair? Another objective is to gain insight into the mechanism of action of EGF induced cell membrane protein phosphorylation in the gut of normal adult mice and those treated with irradiation.

These studies have relevance for industrial toxicology, gastroenterology, immunology, and endocrinology.

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MOLECULAR MECHANISMS OF DIKETONE NEUROTOXICITY

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Neurotoxic Disorders
5 R01 OH01972-02
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$43,581 ($87,563 Cum)

Objectives

n-Hexane and the related solvent methyl n-butyl ketone (MnBK) are neurotoxic in man and a number of experimental animal species. These compounds undergo metabolic conversion to the γ-diketone 2,5-hexanedione (2,5-HD), which is believed to be the active agent in this neuropathy. All γ-diketones tested to date induce a similar neuropathy, while non-γ-diketones are without effect. Other characteristic observations include the appearance of neurofilament (NF)-packed swellings in the distal region of PNS and CNS fibers, covalent tissue protein binding of the compounds, and an apparent direct action upon one or more axonal components.

n-Hexane and MnBK enjoy widespread industrial usage in the furniture, paint, adhesive, and textile industries, and NIOSH has estimated that over two million workers per year receive exposure at or near the current TLV of these solvents. These compounds have been responsible for many instances of human neurotoxic disease in industrial settings, and therefore represent a significant occupational hazard. The hexane-based solvents also serve as models for a large group of industrial chemicals capable of producing a similar human neurotoxic syndrome, including acrylamide, carbon disulfide, and dimethylaminopropionitrile.

Previous studies in this laboratory demonstrated the in vitro and in vivo formation of 2,5-dimethylpyrrole adducts from the reaction of 2,5-HD with protein amine functions. Since only γ-diketones can form the pyrrole derivative, this reaction could account for the observed structure/activity relationships. In addition, autoxidative reactions subsequent to pyrrolylation were found to yield covalently crosslinked protein species. Based on these findings, we have proposed pyrrolylation of neurofilaments as the primary initiating event in γ-diketone neuropathy. The following specific aims are being addressed in this research program: 1) Elucidation of the degree and sites of pyrrole formation in NF proteins following in vitro and in vivo exposure to γ-diketones; 2) Characterization of the physicochemical changes in NF protein structure induced by pyrrolylation; 3) Characterization of the autoxidative reactions occurring secondary to pyrrole adduct formation.

Methodology

Following in vitro or in vivo exposure to 2,5-HD (radiolabelled or unlabelled), axonal cytoskeletal protein preparations are analyzed for pyrrole content by several methods, including colorimetric assay, liquid scintillation counting, and autoradiography of polyacrylamide gels. Physiocochemicals alterations are examined by PAGE, reversed-phase HPLC on octyl-Sepharose, and sedimentation analysis. Sites of pyrrole formation in individual NF subunit proteins are determined by means of specific cleavage with chemical and enzymatic reagents followed by analytical peptide mapping by HPLC or PAGE. Western blotting strategies with anti-MW protein bands present in PAGE gels of axonal cytoskeletal proteins following 2,5-HD exposure. Bovine serum albumin (BSA) is used as a model protein for elucidating chemcial aspects of the pyrrole-forming and secondary autoxidative reactions.

Progress & Accomplishments

In keeping with the original goals of this project, major progress in FY 85 was made in the areas of pyrrole adduct quantitation in axonal cytoskeletal protein preparations from 2,5-HD-treated rats and in chemical studies on pyrrole autoxidation. Evidence for both a time-dependent increase in pyrrole concentration and the formation of crosslinked proteins was obtained in brain stem and spinal cord preparations. These changes were partially reversed upon cessation of exposure, and could be duplicated in similar preparations in vitro. The NF (H) and NF (M) subunit proteins appeared particularly vulnerable to pyrrolylation, since their relative levels decreased in a time-dependent fashion. Actual pyrrole concentrations in the NF subunit proteins were estimated to be 0.5-2 adducts/molecule. In vitro studies using pyrrolylated BSA indicated that the secondary autoxidative process is manifested in several ways, i.e. a decrease in p-diethylaminobenzaldehyde-detectable pyrrole, intermolecular and intramolecular crosslinking, and an increase in absorbance at 475 nm. These phenomena could be inhibited by incubation under N_2 or with ascorbate, and enhanced by the presence of a free-radical initiator (K^+-per-sulfate). The results indicate that the autoxidative phenomena proceed via a free-radical mechanism. Other in vitro studies demonstrated that the intermolecular crosslinking pathway is mediated solely by pyrrole-pyrrole linkages.

Significance

The findings described above represent the first direct evidence for pyrrole adduct formation and covalent crosslinking of NF proteins in γ-diketone-treated animals. Evidence has also been provided to support the hypothesis that the neurotoxic diketones act directly upon axonal cytoskeletal components. Thus, the cytoskeleton in general and neurofilament in particular may be the critical target sites for the neurotoxic diketones. Additional progress has also been made in determining whether pyrrole formation per se is sufficient to induce γ-diketone neuropathy or if additional autoxidative reactions are required. It is hoped that findings derived from this project will ultimately lead to elucidation of the molecular mechanism of action of this important class of neurotoxic chemicals.

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MECHANISMS OF OCCUPATIONAL NEUROPATHIES

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1 R01 OH02003-01A1
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Objectives

The overall goal of this project is to elucidate the mechanisms of organophosphorus compound-induced delayed neurotoxicity (OPIDN). OPIDN has occurred in factory workers exposed in production of organophosphorus chemicals, which are used as plasticizers, lubricants, fire-retardants, or pesticides. Certain animal species (i.e., cats, dogs, cows, and chickens) are also susceptible, while others (rodents and some primates) are less sensititive. It is believed that the initial event in the mechanism of OPIDN is the phosphorylation of a protein, (e.g. neurotoxic esterase, NTE) at the neurotoxicity target. We plan to test the hypothesis that subsequent changes in cellular Ca^{2+} content leads to either (1) an enhanced Ca^{++}-calmodulin-dependent phosphorylation of tubulin, which as a result is aggregated into soluble filamentous polymers distinct from microtubules or (2) an increased Ca^{2+}-activiated proteolytic destruction of neurofilaments. Accumlation or dissolution of such cytoskeletal structures leads to the disruption of axoplasmic transport and the accumulation of mitochondria at the distal parts of the axon. Broken down mitochondria release their calcium stores in the axoplasm. This overloads and disrupts any still functional axonal membrane control mechanisms for intracellular/extracellular ionic gradients, which leads to focal internodal swelling and Ca^{2+}-dependent proteolysis, followed by focal degeneration that spreads somatofugally to involve the entire distal axon.

Methodology

These studies into the mechanisms of OPIDN are being carried out in the hen, the experimental animal for OPIDN, and the cat which may be a better model for extrapolation to humans. The studies include characterization of neurotoxic esterase in the nervous system using SDS-polycrylamide gel electrophoresis (SDS-PAGE); the effect of delayed neurotoxic organophosphorus esters, TOCP, EPN, DFP in comparison with the non-delayed neurotoxic parathion on the normal phosphorylation mechanisms of brain and spinal cord synaptosomal cytosol membrane using [γ^{32}P]ATP and on Ca^{2+} concentration of the axoplasm of neuronal organelles; the isolation and characterization of tubulin and neurofilaments. The effect of delayed neurotoxic organophosphorus compounds on slow and fast axoplasmic transport of radiolabeled proteins and NTE will be investigated.

Progress & Accomplishments

Several studies have been carried out to characterize neurotoxic esterase in the nervous tissues in cats and chickens. NTE has the following characteristics: a) is a membrance bound protein, b) has molecular weight of 155,000 - 178,000 daltons, c) its target size determined by radiation inactivation is 105,000 daltons, and d) it is transported in the sciatic nerve of hens at a fast axonal transport rate of about 150 mm/day. The optimal conditions for the endogenous phosphorylation of brain and spinal cord cytosolic and membrane proteins with [α ^{32}P]ATP were established in hen and compared with those in the rat. The effect of a single oral 750 mg/kg dose of TOCP on the endogenous phosphorylation of brain and spinal cord proteins was assessed in hens during development and recovery from delayed neurotoxicity. TOCP administration confered calcium and calmodulin dependence on the phosphorylation of a few brain cytosolic proteins and caused an increase in the phosphorylation of a number of other cytosolic and membrane proteins. This effect of TOCP was large in magnitude, and in time course reflected the onset of and recovery from the signs of ataxia and paralysis associated with delayed neurotoxicity in the hen. The molecular weights (M_r) and maximal phosphorylation (per cent of control) for the most prominently affected bands were as follows: brain cytosol - 50K (183%), 55K (575%), 60K (529%), 65K (273%), and 70K (548%); brain membranes - 50K (622%) and 60K (697%); and spinal cord cytosol - 20K (182%).

Significance

The present results support the hypothesis that in OPIDN there is an increased phosphorylation of tubulin proteins. The possible causes of this increase is one or more of the following: 1) inactivation of ATPase thus making more ATP available for phosphorylation, 2) decrease in phosphatases activity resulting in less dephosphorylation, 3) more phosphorylation sites available, and or 4) alteration in protein kinase by increasing amount of enzyme or its activity. These possibilities are being investigated. The consequences of altered protein phoksphorylation by delayed neurotoxic organophosphorus compounds are:

1. Increased phosphorylation of MAP-2 depolymerizes cold labile microtubules.
2. Increased phosphorylation of tubulin by ATP results in aggregated polymers.
3. Increased phosphorylation of neurofilaments, tubulin, and MAP2 by Ca^{2+}-calmodulin activated protein kinase may interfere with their ability to form normal interactions.

Publications

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ACRYLAMIDE NEUROTOXICITY: ROLES OF OXIDATIVE METABOLISM

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$56,162 ($109,653 Cum)

Objectives

We are testing the hypothesis that acrylamide (ACR) monomer, a neurotoxic chemical which produces a central-peripheral distal axonopathy, reacts with enzymes essential in oxidative energy transformations thereby compromising the production of high energy phosphates essential for axonal maintenance. Experiments were designed to answer the following questions.

1. Does acrylamide selectively inhibit the activity of oxidative enzymes in neural tissue?
2. Does acrylamide inhibition of glycolytic (previously determined) and/or oxidative enzymes produce a decrease in high energy phosphate levels in peripheral nerves?
3. Is there a correlation between the reduction in axoplasmic transport rates caused by acrylamide and a reduction of high energy phosphate levels?

Methodology

The first question is being approached with a combination of quantitative histochemical and biochemical enzyme assays. Rats were exposed to acrylamide chronically (50 mg/kg/day for 2-10 days) and/or acutely (single 50 mg/kg dose) along with similar animals given saline or methylene-bis acrylamide (MbACR;a non-neurotoxic analogue) in equimolar i.p. injections. Quantitative enzyme histochemical assays for nicotinamide-adenine dinucleotide tetrazolium reductase (NADH-TR) activity in cryostat sections will be performed on specific motoneuron types, dorsal root ganglion neurons, cerebellar Purkinje neurons, hepatocytes, stomach parietal cells and renal convoluted tubule cells. In vitro and in vivo biochemical assays for NADH-dehydrogenase and lipoamide dehydrogenase activity (responsible for the histochemical NADH-TR activity) on these tissues will also be conducted. We will also obtain neuron enriched fractions of cerebral cortex via filtration and filcoll/sucrose gradients and peripheral nerve samples for biochemical enzyme assays. The concentrations of ATP and creatine phosphate in peripheral nerves of saline and acrylamide exposed animals will be measured in control and ACR exposed organelles. Finally, measurements of transport rates and high energy phosphates will be conducted in an attempt to correlate changes with ACR exposure.

Progress & Accomplishments

Initially, the ability of ACR to alter the oxidative enzyme activity (using histochemical NADH-TR activity) in fast glycolytic (low normal activity) and slow oxidative (normally high activity) motoneurons was examined. ACR decreased the enzyme activity 17-20% in slow oxidative motoneurons after 5 and 10 days of exposure. A decrease in retrograde transport of exogenously applied horseradish peroxidase was also observed (ref. 1 below). The acute effects of ACR on NADH-TR activity of these motoneurons demonstrated a 33% inhibition in the slow oxidative motoneurons within one hour of injection; the ability slowly returned over a period of 3 days. Dose-dependent inhibition of the enzymes was demonstrated by adding ACR into the incubation media used to demonstrate NADH-TR activity. The enzyme activity was decreased in a dose-dependent manner from 1-10mM; the maximum inhibition was 54% at 10mM. The non-neurotoxic analogue MbACR was less effective in inhibiting the oxidative enzymes than ACR both in vivo and in vitro. For example, the NADH-TR activity of slow oxidative motoneurons was decreased only 6.7% compared to the 33.4% inhibition by acrylamide (similar results in DRG and Purkinje neurons). In vitro inhibition by MbACR was not dose dependent and was a maximal 14% at 3mM (ref 2 below). Testing for selective action of ACR upon neural enzymes appears promising. With histochemical methods we have found inhibition of NADH-TR in dorsal root ganglia (19.8%;n=4) and cerebellar Purkinje neurons (16.9%;n=7) by ACR while this chemical caused no significant changes in stomach (4.76%), liver (5.53%) and kidney (-3.7%). Unexpectedly, MbACR also significantly inhibited DRG (21.3%) and Purkinje (10%) NADH-TR activity with no effect on the non-neural tissues (paper in preparation). In vitro biochemical assays for NADH dehydrogenase and lipoamide dehydrogenase activity in tissue homogenates and neuron-enriched fractions of cerbral cortex have yielded no effect of ACR on these enzymes. Preincubation of the homogenates with acrylamide at 37C resulted in significant losses of enzyme activity by tissue proteases. We are currently conducting in vivo biochemical assays on tissues from acutely dosed animals.

Significance

Our results have shown that ACR, in doses which produce the central peripheral distal axonopathy, is capable of inhibiting certain enzymes of oxidative metabolism. Our results suggest this is a direct inhibition that occurs in a dose-dependent manner. This suggests that the oxidative enzymes may be the critical site of action in producing the neuropathy. It also explains the ineffectiveness of pyruvate in preventing the neuropathy; the oxidative enzyme inhibitions are distal in the energy transformation pathways to the exogenous substrate. We are further encouraged by the lower effectiveness of MbACR in inhibiting these enzymes, further emphasizing the importance of our findings. The neural selectivity of ACR may be due to inactivation of neuron specific isoenzymes. Our data suggests that neuron specific forms of NADH-dehydrogenase and/or lipoamide dehydrogenase exist and are specifically inhibited by ACR. It is essential that high energy phosphates are measured to ensure these enzyme inhibitions are meaningful.

Publications - Abstracts available

Sickles DW, Goldstein BD: Acrylamide alters oxidative enzyme activity of rat motoneurons. Toxicology Letters 26:111-118, 1985.

MECHANISMS OF OCCUPATIONAL DISTAL AXONOPATHY

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Neurotoxic Disorders
1 R01 OH02065-01
05/01/85 - 04/30/88
$131,882 ($131,882 Cum)

Objectives

These studies are designed to further understanding of how neurotoxic agents alter normal neuronal processes to produce axonopathy. Central-peripheral distal axonopathy is produced in laboratory animals repeatedly dosed with acrylamide and is a useful model of neuronal degenerative disease. Acrylamide neurotoxicity may be initiated by an inhibition of the retrograde axonal transport system which interrupts the flow of information from axon to perikaryon. As a consequence, the neuron fails to mount the appropriate repair responses when challenged distally and the axon undergoes distal-proximal degeneration. To test this novel hypothesis we are measuring markers of repair-associated gene expression following axotomy in the presence and absence of systemic acrylamide intoxication.

Methodology

The bipolar sensory neurons of mouse dorsal root ganglia and their projections located in the sciatic nerve constitute the *in vivo* model system employed to study perikaryon repair responsiveness. Experimental protocols are designed to study the inhibitory effects of acrylamide and other agents including colchicine and actinomycin D on the increases in perikaryon RNA polymerase activity (an index of repair response) following transection of the sciatic nerve.

Progress & Accomplishments

Recent progress includes the development of assay methods to measure RNA polymerase activity and the DNA content of mouse dorsal root ganglia. Data have demonstrated that following transection of the sciatic nerve, RNa polymerase activities are significantly elevated in dorsal root ganglia by day 2 post-axotomy. Preliminary experiments with several dosing regimens of acrylamide reveal that this agent can attenuate the increase in RNA polymerase activity which follows axotomy. When taken in concert with earlier experiments which demonstrated that single and repeated doses of acrylamide cause a reduction in the rate of retrograde axoplasmic transport, these results provide strong support for our hypothesis of altered repair responsiveness in acrylamide-induced neurotoxicity.

Significance

Monomeric acrylamide is an environmental and occupational neurotoxin which causes polyneuropathy in humans. Despite many years of intensive research, the exact toxic mechanism of acrylamide remains unknown. Diminished perikaryal repair capacity, in the presence of low-level axon damage, represents a novel mechanism by which toxic agents may alter neuronal function or structure. These studies provide detailed data regarding the means by which neuronal repair responses are initiated and regulated, substantially adding to our understanding of the mechanisms by which neurotoxic agents produce axonopathy.

__Publications__

Miller M, Spencer PS: Mechanisms of acrylamide axonopathy. Annual Review of Pharmacology and Toxicology p 643-666, 1985.

Spencer PS, Arezzo JC, Schaumburg HH: Chemicals causing disease of neurons and their processes. Neurotoxicity of Industrial and Commercial Chemicals CRC Press, Boca Raton, p 1-4, 1985.

Spencer PS, Sabri MI, Moretto A, Miller M, Soiefer A: Chemical probes of neuroaxonal dystrophy. Int Conf Neuroaxonal Dyst Feb, 1986.

VALIDATION OF A NEUROBEHAVIORAL TEST BATTERY

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Neurotoxic Disorders
1 R01 OH02157-01
09/27/85 - 08/31/87
$157,787 ($157,787 Cum)

Objectives

To validate a computer-administered neurobehavioral test system for use in field situations for detecting the intoxicating effects of solvents.

Methodology

Transient changes in central nervous system (CNS) function will be assessed using a computer-administered neurobehavioral evaluation system (NES). Industrial hygiene sampling, exhaled breath analysis, and urine analysis will be coupled with biomathematical dose models to estimate CNS concentrations of solvent. To reduce the complexity of exposure estimation, we will study fiberglass boat-builders, exposed almost exclusively to styrene. NES testing will be performed before and after exposure to styrene during the work day.

Progress & Accomplishments

Newly awarded Grant.

Significance

We anticipate that this study will demonstrate the utility of our NES in assessing the intoxicating effects of solvents in the field study situation and provide information directly relevant to the setting of workplace exposure standards. It should improve our ability to monitor exposure and effect and to establish dose-effect relationships in solvent-exposured workers.

Publications - None to date

PERIPHERAL NERVOUS EFFECTS OF WORKPLACE NEUROTOXINS

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Neurotoxic Disorders
5 K01 OH00043-02
09/28/34 - 08/31/87
$32,400 ($64,800 Cum)

Objectives

To develop methods that are accurate, sensitive, and efficient for field evaluation of peripheral nervous system functions in workers exposed to neurotoxic agents.

Methodology

Three aspects of peripheral nervous system function have been selected for study: tremor, temperature sensitivity and vibration sensitivity. The project consists of five phases: (1) Examination and modification of test apparatus and data collection procedures, (2) reproducibility testing (3) normative data development (4) testing of exposed populations and (5) development of protocol manual.

Progress & Accomplishments

For tremor testing, peripheral equipment has been assembled and computer software integrated to allow acquisition and storage of tremor signals as well as performing Fast Fourier Transforms of signals for frequency analysis. For temperature and vibration, instruments from Sensortek, Inc., have been selected and tested. We have implemented a procedure for estimating sensory thresholds that is more efficient than that proposed by the instrument manufacturer. We have performed computer simulations that show our procedure to be more accurate and to take less time than the manufacturer's suggested protocol.

We have collected data using our new protocol on more than 110 unexposed workers for development of norms for both the temperature and vibration a test devices. In addition, we have collected sensory test data during field studies of solvent exposed painters. 113 painters have been tested with the temperature device and 55 have been tested with the vibration device. Exposure-effect analyses on these data, as well as continued data collection for reliability and stability of these measures are in progress.

Significance

These tests and new procedures should expand our ability to study the effects on the PNS of neurotoxic agents in the workplace.

Publications - None to date

SYMPTOMS OF NEUROLOGIC DISEASES IN CONSTRUCTION PAINTERS

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Neurotoxic Disorders
1 R03 OH01704-01
07/01/83 - 08/31/85
$19,736 ($19,736 Cum)

Objectives

To assess whether construction painting with solvent-based paints was associated with excess risks of symptoms of peripheral neuropathy, symptoms of depression, and neurasthenic symptoms.

Methodology

A sample of 4,118 painters was selected from the membership of the International Brotherhood of Painters and Allied Trades, stratified by union local, geographic region, and year of birth. A similar stratified sample of 4,121 glaziers was selected from the same Union as a comparison group exposed little, if at all, to solvents. Exposure to solvent-based paints was assessed by a series of questions about work experience developed by the Union. Symptoms were assessed by means of a self-administered postal questionnaire which contained the Center for Epidemiologic Studies Depression Scale; Questionnaire 16, a list of neurasthenic symptoms; and a peripheral neuropathy scale developed and validated specifically for this study.

Progress & Accomplishments

The response rate to the mailed questionnaires was 34.0 per cent for painters and 36.4 per cent for glaziers. On the basis of answers to the questionnaire, 994 respondents were classified as painters and 1218 as glaziers. Interior painting was significantly associated with symptoms of depression, and industrial painting was associated with neurasthenic symptoms. Both types of symptoms appeared to be related to exposure to solvent-based paints. Glaziers had an excess of symptoms of peripheral neuropathy, probably related to ergonomic factors in the process of glazing.

The study has been completed. A full report has been sent to the Union, and the data formed the basis for a doctoral thesis for Richard W. Freeman, submitted to the School of Hygiene and Public Health after a successful defense in September, 1985.

Significance

This study indicates that there is a risk of depressive and neurasthenic symptoms associated with the use of solvent-based paints, especially in industrial interior and exterior, and residential interior applications. It points up the needs to ascertain which solvents are most likely to produce these symptoms and to evaluate protective procedures. It also suggests that there may be hitherto unsuspected hazard in glazing, namely peripheral neuropathy and particularly suspected nerve entrapment at the wrist. Further investigation of this potential hazard and methods of prevention is needed.

Publications

Freeman RW, Bleecker ML, Comstock GW, Brookmeyer RS: Validation of self-administered questionnaire for study of peripheral neuropathy. American Journal of Epidemiology 121:291-300, February 1985.

Freeman RW: A survey of neurologic symptoms among members of the International Brotherhood of Painters and Allied Trades. Doctoral dissertation, submitted to the Johns Hopkins School of Hygiene and Public Health, Baltimore, MD, September, 1985.

NEUROTOXIC ESTERASE AND NEUROTOXICITY

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Neurotoxic Disorders
5 R03 OH01761-02
09/01/83 - 08/31/86
$28,125 ($54,585 Cum)

Objectives

Single exposures to certain organophosphorus compounds (OP) induce delayed onset of peripheral neuropathy which is associated with distal axonal degeneration of peripheral nerves and long spinal tracts. The initial event, occurring within hours of dosing, is the phosphorylation and "aging" of a target protein (NTE: Neuropathy Target Esterase), but the relationship between this event and the delayed onset of axon degeneration is unknown. The objective of this study is to determine whether the OP-NTE reaction is followed by detectable pathophysiological changes which precede the onset of the first morphological or neurobehavioral abnormality in the test animal (hen). Specific focus has been placed on the fate on retrograde axon transport since this critical system appears to be exceptionally vulnerable in toxic neuropathies.

Methodology

Current studies are examining the relationship between NTE/OP interaction and the fate of retrograde transport of ^{125}I-tetanus toxin (a marker protein) in hen sciatic nerve after a single, subcutaneous injection of either neurotoxic (DBDCVP) or non-neurotoxic (PMSF or paraoxon) compounds. At selected intervals after receiving one of these compounds, animals are administered a single intermuscular (gastrocnemius) injection of ^{125}I-tetanus toxin and retrograde axonal transport of radiolabel is subsequently assessed by determining the content of ^{125}I that has been transported to lumbar dorsal root ganglia (sensory axons) and ventral spinal cord (motor axons). Chickens treated with comparable doses of PMSF or paraoxon are used as controls to determine if defective transport is related to NTE inhibition/"aging".

Acronyms DBDCVP: Di-n-butyl-2, 2-dichlorovinylphosphate, a neuropathic agent which inhibits/"ages" NTE and also inhibits acetylcholinesterase (unrelated to neuropathy induction); PMSF: Phenylmethanesulfonylfluoride, a non-neuropathic agent which inhibits but does not "age" NTE; Paraoxon: Diethyl-p-nitrophenylphosphate, an agent which inhibits acetycholinesterase only.

Progress & Accomplishments

Single injections of neurotoxic doses of DBDCVP, an OP compound which in these amounts induces the appearance of neuropathy in the hen after 1-2 weeks, results in a time-dependent decrease in accumulation of ^{125}I in both ventral spinal cord and dorsal root ganglia. Neither PMSF or paraoxon has a significant effect on the retrograde transport of the radiolabel. At 7.5 days after intoxication, when pathological and functional evidence of neuropathy is lacking, the accumulation of radiolabel is reduced approximately 70% in dorsal root ganglia and 65% in ventral spinal cord.

These studies demonstrate that changes in retrograde axonal transport precede the onset of peripheral neuropathy. Deficits in axon transport are significant by 2 days following injection of the neurotoxic compound, and these deficits increase in severity until axon degeneration commences. This result is comparable to an earlier study with acrylamide (work funded on other NIOSH grants) in which changes in axon transport, visible as early as 6 hours following a single dose, accrued with repetitive doses until the onset of nerve fiber degeneration and appearance of peripheral neuropathy. Acrylamide had no effect on the uptake of ^{125}I-tetanus toxin. If altered uptake of radiolabel can be ruled out for the test OP compound, this would provide evidence that two chemically unrelated axonopathic agents (DBDCVP and acrylamide) mediate their neurotoxic effects by interfering with retrograde axon transport. The precise function of this system is unknown but it is likely to include the return of axoplasmic elements to the neuronal perikaryon for "re-processing" and the communication of information on axon integrity to the perikaryon. Although the mechanism by which DBDVCP inteferes with retrograde transport is unknown, these experiments suggest that the mechanism is linked to the inhibition/"aging" of NTE, an axonal protein. This conclusion will be strengthened if subsequent studies show that pretreatment with PMSF, which blocks OP-induced "aging" of inhibited NTE and onset of neuropathy, also prevents DBDVCP-induced alterations in retrograde axon transport.

Significance

Peripheral neuropathy is a remarkably common response of the human nervous system to systemic intoxication with occupational chemicals, including certain OP compounds, acrylamide, n-hexane, methyl n-butyl ketone, carbon disulfide, and dimethylaminopropiontrile. These neuropathies are all associated with distal axon degeneration of long and large-diameter nerve fibers in peripheral nerves and long tracts of the spinal cord. The mechanisms underlying axonal degeneration in OP neuropathy are not understood, but the results of the current study and other data from this laboratory suggest that alterations in retrograde axonal transport may play an important role in the pathogenesis of nerve fiber degeneration in this and related toxic neuropathies.

Publications - Abstract(s) available

Spencer PS, Arezzo JC, Schaumburg HH: Chemicals causing disease of neurons and their processes. In: Neurotoxicity of Industrial and Commercial Chemicals (ed. O'Donoghue, J.), CRC Press, Boca Raton, p 1-4, 1985.

Spencer PS, Miller MS, Ross SM, Schwab B, Sabri MI: Biochemical mechanisms underlying primary axonal degeneration. In: Handbook of Neurochemistry (ed. Lajtha, A.), vol. 9., Plenum Press, New York, p 31-65, 1985.

NEUROPHYSIOLOGY OF ORGANOPHOSPHORUS NEUROTOXICITY

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Neurotoxic Disorders
5 R03 OH01763-02
07/01/83 - 06/30/85
$22,478 ($44,952 Cum)

Objectives

The overall objective of the proposal was to investigate the neurophysiological and neurological effects or tri-o-cresyl phosphate induced delayed neuropathy in the cat. The changes were to be correlated with the histological changes which occur.

Methodology

In this study, cats were treated with doses of 100, 250, 500, 1000, 1500, and 3000mg/kg. Cats were examined for neurological, electromyographical and histopathological changes. In addition, electrophysiological studies of cats administered either a single dermal dose of 1500 mg/kg TOCP or two doses of 500 mg/kg TOCP, 5 days apart have also been done. Monosynaptic reflex responses as well as dorsal and ventral root compound action potentials were recorded. Animals were observed for 60 days after which monosynaptic reflex responses were recorded from L7 or S1 ventral roots after stimulation of the tibial or common pereneal nerves. Post-tetanic potentiated response was also recorded. Nervous tissues from all control and treated cats were examined histopathologically.

Progress & Accomplishments

Neurological and histological examinations of cats treated with TOCP have been completed. Neurologic signs of peripheral and central nervous system deficits were readily apparent in cats treated with dermal doses of greater than 250 mg/kg TOCP. Electrophysiological studies of cats administered TOCP have also been done. There was a significant decrease from control of the unconditioned MSR. The post-tetanic potentiated response was significantly decreased in the S1 ventral root, however when expressed as a percent as a factor of potentiation of the unconditioned MSR, it was found to be increased albeit not significantly. The dorsal and ventral root compound action potentials were also decreased in amplitude from control although the conduction velocities were unaltered. The decrease in the unconditioned MSR, as well as the ventral and dorsal root compound action potentials were attributed to peripheral nerve damage. The absence of any significant change in the post-tetanic potentiated response expressed as a factor of potentiation of the unconditioned MSR was attributed to a decrease in both the discharge zone as well as the subliminal fringe. The results of the neuromuscular junction transmission and peripheral nerve function tests showed no changes in repetitive stimulation motor nerve conduction study (RS-MNCS) or sensory nerve conduction study (SNCS) for all cats treated with a single, dermal dose to TOCP. EMG abnormalities were observed in cats treated with a single, dermal dose of 250 to 1,500 mg/kg TOCP, but not those cats given 100 mg/kg TOCP. Severity and and frequency of EMG abnormalities in tested cats were dose dependent. EMG changes consisted of positive waves and fibrillation in a setting of increased insertional activity in the muscles of the hindlimb at rest; this is in contrast to the normally silent state of resting muscle. A comparison of the electromyographic abnormalities associated with dosage and the onset of neurologic signs of TOCP delayed neurotoxicity showed that clinical deficits always preceeded EMG changes. Appearance of EMG abnormalities took place concurrently with the severe ataxia stage (N_3). Also, as the clinical condition of treated cats improved, the abnormal, spontaneous activity disappeared despite persistence of a lesser grade of neurologic dysfunction associated with single or daily administration.

Histopathologic lesions in spinal cord and peripheral nerves were characterized by central-peripheral distal axonopathy degeneration. These lesions were confined to the longest tracts and were initially present, in the cats that died early, in the most distal portion of the tracts affected in both central and peripheral nervous systems.

Spinal cord. No histopathological lesions were seen in the cats that died 6 and 7 days following the administration of a single, dermal dose of TOCP. Early histopathologic changes were seen in the spinal cord of the cat that died after 25 days. These changes were swelling, vacuolation, and occasional fragmentation of axons and myelin sheaths. The changes were concentrated in the gracile and spinocerebellar ascending tracts and posterior columns of the upper cervical levels. The lateral corticospinal descending tracts were damaged in throacic and lumbar spinal cord. The anterior, presumably descending, fibers were most severely damaged in the lumbar region. Degeneration was also present at lumbar levels in tracts lying in the ventral fissure. Neuronal cell death was not seen. Cellular lesions were characterized by Wallerian-type degeneration of axons and myelin sheaths and were observed most consistently in thoracic spinal cord. Spinal cord tissues of cats killed at the end of the experiment exhibited more damage in these long pathways. Also, severity and frequency of the lesions in spinal cords of treated cats depended on the size of the applied dose. Histopathologic changes were most severe in all examined cats given 1,500 and 1,000 mg/kg TOCP. Two of the cats given 500 mg/kg and all three dosed with 250 mg/kg showed histologic lesions. Individual differences among test animals and/or sampling may account for the absence of neuropathologic lesions in one cat given 500 mg/kg TOCP. Extensive axonal degeneration and phagocytic cells were seen in the longitudinal sections of the anterior columns in the thoracic spinal cord from cats given a single 1,000 mg/kg dose of TOCP. A completely degenerated axon was seen in the longitudinal section of the thoracic spinal cord from a cat given 250 mg/kg TOCP.

Peripheral nerves. In this study the peripheral nerves from the spinal cord down to the level of the ankle in treated and control cats were examined. Histopathologic lesions were not detected in the sciatic nerve, but were easily seen in the peripheral branches. Severity and frequency of these lesions were also dependent on the dose and progression of clinical signs.

Finally, data from control and TOCP treated cats has been obtained on the stimulas bound repetition. The results to data are inconsistant, particularly from the treated animals. We are currently continuing with this project in order to obtain enough data to make statistical comparisons between control and treated animals.

Significance

The data obtained from the other studies mentioned above, gives one the ability to intergrate electrophysiological, neurological and pathological findings into a much more coherent view of TOCP induced neurotoxicity in a mammalian species, and thus a greater understanding of organophosphorus induced delayed neurotoxicity in the human.

Publications

Abou-Donia MB, Jensen DN, Lapadula DM: Neurologic manifestations of tri-o-cresyl phosphate delayed neurotoxicity in cats. Neurobehavioral Toxicology and Teratology 5:431-442, 1983.

NEUROBEHAVIORAL EFFECTS OF EXPOSURE TO ORGANIC SOLVENTS

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Neurotoxic Disorders
1 R03 OH01799-01A1
08/01/84 - 07/31/85
$19,125 ($19,125 Cum)

*Presently at NIOSH/DSHEFS, 4676 Columbia Parkway, Cincinnati, Ohio 45226

Objectives

Chronic exposure to mixed organic solvents is known to affect adversely the functioning of the central nervous system (CNS). Chronic CNS disorders reported in association with exposure to solvents and other toxic agents have been classified into three major types in a scheme adopted by the World Health Organization. The earliest form of chronic toxicity, referred to as a Type 1 effect, consists primarily of an increase in symptoms, such as sleep disturbances, fatiguability, and difficulty concentrating. More severe than the presence of symptoms alone is mild chronic toxic encephalopathy, characterized as either an organic personality or mood disorder (Type 2a) or a disorder of deficits on neurobehavioral functions, such as psychomotor and visuo-perceptual abilities, attention, and short-term memory (Type 2b). A Type 3 disorder, severe chronic toxic encephalopathy, resembles other forms of permanent dementias.

The primary objective of this study was to determine the nature and extent of neurotoxic effects of exposure to mixed organic solvents among a group of construction and maintenance painters. Specific aims were 1) to evaluate the field applicability of a computerized set of neurobehavioral tests (Neurobehavioral Evaluation System), which included measures of reaction time, attention, hand-eye coordination, verbal abilities, and short-term memory; 2) to evaluate the Sternberg memory-scanning task as a field test of short-term memory; and 3) to measure the occurrence of neurotoxic symptoms and determine their correlation with measures of sub-clinical neurobehavioral deficits.

Methodology

A cross-sectional study of 101 construction painters was peformed, where exposure to solvents was estimated using questionnaire data to derive an Exposure Index (measure of intensity of exposure), as well as duration and frequency of exposure. A control group of dry wall tapers was also tested. However, because of doubts about their comparability with the painters, all analysis reported are internal comparisons within the group of painters. Adverse CNS effects were assessed by self-reported questionnaires and eight tests of the computer-administered Neurobehavioral Evaluation System (NES) (continuous performance test (CPT), symbol-digit substitution, hand-eye coordination, digit span, pattern memory, Sternberg memory scanning, vocabulary, and mood) (Baker, et al, JOM 27: 206-212, 1985). In addition, a comprehensive health questionnaire was administered to assess previous health problems, medication history, and other potential confounders, such as alcohol and caffeine use. Data analysis included factor analyses to provide appropriate groupings of neurobehavioral tests and, separately, symptom reports. Multiple regression techniques were used to investigate the relationship between solvent exposure and measures of effect. In addition to measures of exposure, independent variables included age, years of school, vocabulary score, and estimates of alcohol intake and socio-economic status.

Progress & Accomplishments

The validity of the NES tests and symptom reports was supported by the results of the factor analyses which yielded factors, in each case, that were both biologically plausible and in agreement with other empirical evidence. This study provides substantial evidence of a statistically significant relationship between several measures of exposure to solvent paints and the occurrence of neurotoxic symptoms, including dizziness, nausea, fatigue, problems associated with arm strength, and the feeling of getting "high" from solvent-containing products. However, very few significant positive associations with exposure were found among tests of neurobehavioral function. Most notable of these were the associations between the number of weeks worked with solvents in the previous year and symbol-digit latency ($p=.04$) and digit span (forward) ($p=.03$).

The parameters of interest of the Sternberg memory scanning test are the intercept, a measure of cognitive and motor processing time, and the slope, an estimate of memory scanning time. Not surprisingly, the intercept correlated more highly with measures of psychomotor function (CPT: $r=.39$; symbol-digit latency: $r=.31$ ($p < .001$ for each) that with other measures of short-term memory (digit span: $r=-.10$; pattern memory: $r=-.02$). The slope did not significantly correlate with any other tests results; the correlation with digit span and pattern memory were relatively low ($r=-.15$ and $-.12$, respectively). In addition, because of the time constraints of a field study, the number of trials administered was modest (total of 240), yielding parameter estimates which were quite unstable. It is likely that stable estimates of both the intercept and slope can only be achieved from a test which is prohibitively long in duration for field applicability.

The correlation of symptom factors and NES tests showed that any significant relationships were limited to the association of symptoms with Mood Scale scores (modification of Profile of Mood States). There were no remarkable associations or patterns of association seen among the other behavioral tests and reports of neurotoxic symptoms.

Significance

The pattern of the toxic effect of mixed organic solvents on neurobehavioral function demonstrated in this population, i.e. the presence of neurotoxic symptoms without clear functional deficit, is consistent with Type 1 effects as categorized by the WHO. The National Institute for Occupational Safety and Health (NIOSH) has identified neurotoxic disorders as one of the ten leading problems in work-related disease and injury. This research provides evidence of an early, potentially reversible chronic toxicity among construction painters.

Publications - None to date

OCCUPATIONAL ETIOLOGIES OF ALZHEIMER'S DISEASE

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Neurotoxic Disorders
1 R03 OH01826-01A1
12/01/84 - 5/31/86
$24,450 ($24,450 Cum)

Objectives

Evaluate the hypothesis that Alzheimer's disease or a closely related clinical dementia are related to occupational exposure to organic solvents and/or lead.

Methodology

A case-control study of clinically diagnosed cases of Alzheimer's disease was conducted to evaluate occupational exposure to organic solvent and/or lead as potential risk factors. Neighborhood controls were selected from the list of registered voters for Massachusetts and were matched by sex, year of birth, and town of residence Occupation was assessed by mailed questionnaire sent to spouses or next of kin residing in the same household. An exposure index was utilized to assess exposure to organic solvent. A conditional logistic regression analysis was performed, odds ratios and two-sided 95% confidence intervals were calculated.

Progress & Accomplishments

Detailed occupational histories were gathered from spouses and next of kin for 106 cases and 214 controls. A conditional logistic regression analysis was conducted for 98 cases and their 162 matched controls. After controlling for educational level no apparent association was observed between risk of Alzheimer's disease and occupational exposure to organic solvent and/or lead.

Significance

Prior scandinavian studies have noted an excess of presenile dementia and encephalopathy in workers exposed to organic solvents and/or lead. The current study suggests that this condition may not be identical to DSM III criteria dementia of the Alzheimer's type. Further study of dementias not specifically of the Alzheimer's type may be in order.

Publications - None to date

NEUROBEHAVIORAL EFFECTS OF BRIEF EXPOSURE TO LEAD

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Neurotoxic Disorders
1 R03 OH01893-01
04/01/84 - 03/31/85
$18,028 ($18,028 Cum)

Objective

This study was carried out to assess whether central nervous system (CNS) impairment, particularly memory and learning impairment, are early (less than one year) effects associated with lead exposure in adults.

Methodology

Thirty male lead workers were evaluated by comparing their performance on a neurobehavioral test battery to that of 60 control workers. Controls were matched two-to-one to lead workers on age, sex and education level. The lead exposed men had worked for one year or less in jobs which required burning lead-based paint. They ranged in age from 18 to 60 years with a medium age of 34.

Blood lead concentration and whole blood erythrocyte protoporphyrin levels levels were used to assess lead exposure in both groups. Analyses were performed in a CDC reference laboratory. Blood level was measured by a modified Delves cup atomic absorption method and erythrocyte protoporphyrin measurements were made on whole blood using a double extraction method and read on a microfluorophotometer.

The neurobehavioral test battery included 14 subtests designed to evaluate several central nervous system functions. Tests of learning and memory were emphasized in 9 of the subjects. Reaction time, psychomotor speed, visual perceptual ability and attention were also addressed.

Education level and vocabulary test scores were used as indicators of baseline intelligence. Neurobehavioral test scores of lead-exposed men were compared to those of age and education as well as age and vocabulary matched controls.

Data were analyzed for differences in performance between the exposed and control groups and for associations between exposure indices and neurobehavioral measures.

Progress & Accomplishments

The study was completed as of December 1984. A summary of results follows: The two study groups were comparable in sex, age and education level and a measure of baseline intellectual ability, vocabulary. Blood lead concentrations of lead-exposed subjects at the time of testing ranged from 42 to 152 ug/dl with a median of 60 up/dl. Erythrocyte protoporhyrin levels ranged from 44 to 310 ug/dl wih a median of 162 ug/dl. Blood lead levels of controls did not exceed 21 ug/dl and their maximum erythrocyte protoporphyrin level was 36 ug/dl.

Lead exposed workers performed significantly more poorly than controls on three tests: Simple visual reaction time, Rey auditory verbal learning; and a cancellation task which required psychomotor speed, visual scanning and sustained attention. On the learning task, the lead-exposed group achieved lower scores than controls on the last two of five trials ($P<.05$). Analysis of the recognition trial suggested a deficit in encoding or retaining new material. No association was observed between indices of lead exposure, blood lead and erythrocyte protophorphyrin, and test scores.

Significance

Previous studies of lead workers have suggested impairment of neurobehavioral functions. However, these studies have not focused on short-term exposure nor have they conducted extensive evaluations of memory of learning ability. Findings in this study suggest early direct or indirect effects of lead on central nervous system functioning of adults with subacute exposure. A possible learning deficit due to lead exposure has been demonstrated.

Publications - None to date

DETECTION OF POLYCHLORINATED BIPHENYL NEUROTOXICITY

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Neurotoxic Disorders
5 R03 OH01898-02
07/01/83 - 09/30/85
$25,028 ($50,003 Cum)

Objectives

The major objective of this proposal has been the determination of the feasibility of using peripherally obtained biological specimens such as urine to estimate neurological dysfunction caused by acute and sub-chronic exposure to polychlorinated biphenyls (PCBs). These neurological changes have been estimated by determining catechol and indoleamine neurotransmitter and metabolite changes in brain and urine using high-performance-liquid chromatography with electrochemical detection (HPLC-ECD). More specifically, we have determined: (1) the relationship between PCB body burden and central neurochemical change; (2) the relationship between central neurochemical change and neurochemical changes measured in the periphery; (3) the neurotoxicity of Aroclor mixtures that differ in the degree of chlorination and (4) the utility of determining exposure to PCBs by measuring concentrations of these congeners in urine.

Methodology

One of the major goals of this research project has been development of means to detect neurotoxicity induced by exposure to PCBs. The approach has been to expose laboratory rats to commercial mixtures of PCBs that differ in their degree of chlorination and to correlate the central and peripheral neurochemical changes caused by exposure to these PCBs.

These series of experiments have involved acute and sub-chronic exposure of rats to PCBs. 24-h urine collections were made from the animals at designated intervals. Animals were then sacrificed and regional brain catecholoamine and PCBs concentrations were determined. Biogenic amines and their metabolite concentrations in brain and in urine were determined by HPLC-ECD employing recently developed techniques described in J. Chrom. (Biomed. Applic.) in press. PCB concentrations in regional brain sections, plasma and urine were determined by glass capillary high resolution gas chromatography (GC). This design allowed us to correlate urinary metabolite changes with central neurochemical changes and PCB body and brain burdens.

Progress & Accomplishments

Exposure of Wistar-derived rats to mixtures containing equal amounts of Aroclors 1254 and 1260 induced central neurochemical changes including regional alterations in norepinephrine concentrations, Neurotoxicology, 6, (3) 13-24, 1985. We have also noted changes in serotonin concentrations following exposure to these Aroclor mixtures (Neurotoxicology, 7, (1) 155-166, 1986) and changes in striatal and lateral olfactory concentrations of dopamine and its major metabolite homovanillic acid, (HVA) Toxicology Letters, in press, 1986. We also demonstrated that PCBs induce prolonged increases in urinary HVA production that are related to PCB exposure but not to alterations in food or water consumption; (Journal of Toxicology and Environmental Health, 915, 575-586, 1985).

BEST COPY AVAILABLE

Following exposure of rats to equal amounts of lightly chlorinated Aroclor 1016 and heavily chlorinated Aroclor 1260 we have shown by GC analysis of brain tissue that no component of Aroclor 1016 accumulates in brain; whereas some congeners of Aroclor 1260 accumulate to a significant degree. Furthermore, Aroclor 1260 induces central neurochemical changes whereas exposure to Aroclor 1016 does not. GC analysis of brains of animals fed Aroclor 1260 demonstrates that only a few congeners accumulate in brain; (245, 2'3'4'5', 245, 2'4'5', 234, 2'4'5', and 345, 2'3'4'5') and represent more than 50% of the total PCB residue in brain. Because neurochemical change is seen only following exposure to Aroclor 1260 and because these previously mentioned congeners are present in high concentrations in brain, it is likely that these congeners may be responsible for the observed neurotoxicity.

We have also demonstrated that changes in urinary HVA production induced by the more heavily chlorinated congeners are due to changes in brain HVA rather than changes in peripheral production of HVA. We have demonstrated this by exposing rats to either Aroclor 1016 or Aroclor 1260 and measuring HVA production following the administration of the peripheral monoamine oxidase inhibitor debrisoquin sulfate (DS). This compound has been shown to inhibit the formation of HVA but is unable to cross the blood brain barrier. DS resulted in a decreased total production of HVA measured in the periphery but did not alter central formation of this metabolite. Animals exposed to Aroclor 1016 and then given DS show no differences in production of HVA compared to animals that received DS and corn oil. However, animals exposed to Aroclor 1260 and DS continued to show elevated concentrations of urinary HVA, strongly suggesting that the elevation in urinary HVA in the Aroclor 1260 exposed group was due to elevated central production of HVA.

We have also measured PCB congener concentrations in the urine of animals exposed to PCBs and compared them with the PCB congener concentrations found in plasma. The obtained results indicate that determination of PCB congener patterns and concentrations in urine provide a more easily obtainable and accurate estimate of the exposure of the organism to environmental PCBs than that which can be obtained by measurement of PCB concentrations in plasma.

Significance

Application of novel techniques for determining catecholamines and PCBs, together with innovative experimental design and statistical analysis has allowed us to unequivocally demonstrate that exposure of rats to PCBs induces significant changes in several brain neurotransmitters. These results provide a means of quantifying and validating the neurological complaints observed in workers occupationally exposed to PCBs. Furthermore, the PCB concentrations are relevant to the industrial context because of PCB concentrations on the fat of these animals are only 2-3 times those that have been reported in industrial workers exposed to PCBs.

The demonstration that elevated urinary HVA production following exposure to PCBs is due to increased central nervous system (CNS) production of HVA indicates that measurement of HVA in the urine of occupationally exposed workers may provide a useful index of CNS change. Furthermore, we have demonstrated that measurement of PCB congener patterns and concentrations in urine may provide a more readily obtainable and accurate estimate of exposure to PCBs than those which are normally obtained by measurements of PCB congener concentrations in plasma.

Heavily chlorinated congeners found in Aroclors 1254 and 1260 but not the lightly chlorinated PCB congeners found in Aroclor 1016 accumulate in brain following sub-chronic exposure of rats to these complex mixtures, but only the heavily chlorinated congeners induce central neurochemical changes. Furthermore, four congeners present in the Aroclor 1260 mixture make up more than 50% of the PCB brain residue suggesting that the neurotoxicity of complex mixtures may be due to a few congeners. These results suggest that the risk of exposure in the environment may be more adequately predicted by determining the PCB congener patterns of workers or individuals exposed to PCBs in the environment, than by estimating risk based on total PCB levels.

Publications - Abstracts available

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THE EFFECTS OF CADMIUM ON THE RAT OLFACTORY SYSTEM

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Neurotoxic Disorders
5 R03 OH02038-02
09/28/84 - 08/31/86
$2,362 ($17,524 Cum)

Objectives

The overall goal of the project is to determine what effects chronic low-level exposure to cadmium (via inhalation) may have on the rat olfactory system. The hypothesis to be tested is as follows: exposure of rats to chronic low levels of Cd via inhalation results in impairment of the olfactory system, both functionally and structurally.

Methodology

This project will determine whether exposure of rats to chronic low levels of Cd (250, 500 mg CdO/m^3; 5 hr/day, 5 days/week, 80 to 90 days via inhalation alters the odor detection threshold in a dose-related fashion, by using the conditioned suppression technique. The extent of histological alterations of any Cd-induced lesions in the olfactory bulb will be determined by light microscopy. Alterations in any histological parameters will be quantified by Magic Scan analysis. This may provide information as to site(s) of Cd-induced lesions in the olfactory system. This project will also correlate alterations in odor thresholds and histology with Cd content in the olfactory bulb, the latter determined by atomic absorption spectorphotometry. Finally, this project will compare relative toxicity of Cd in the olfactory system with that in the lung and heart.

Progress & Accomplishments

Forty-five rats have been exposed to 0, 250, or 500 mg/m^3 CdO aerosol, 5 hr/day, 5 days/week, for 70 days.

Neither level of CdO aerosol produced any changes in the body weight or in proteinuria. It should be pointed out that weight is controlled (by water deprivation) for those rats undergoing olfactory testing. However, no differences were seen in a subgroup of subjects which were not water deprived.

The Cd content in the kidney (mg/g net weight) is presented in the following table.

	CdO Exposure Time	
	5 Weeks	10 Weeks
Control	.05	.06
250 mg/m^3	2.2	3.8
500 mg/m^3	3.65	5.07

The accumulation of Cd in the kidney is both time - and dose - related. These values are in consistent with those found by Prigge et al (1978).

There has been no difference in the kidney Cd content between water deprived and non water deprived rats, suggesting water deprivation regimen did not affect Cd absorption in the kidney.

The olfactory threshold levels of most rats have remained constant after 70 days of exposure to low levels of CdO. A subset of rats showed a transient decrease in threshold levels as a result of decreased shock threshold levels. The latter might have been due to the variation of the equipment or the weather.

Significance

Cd content in the kidney cortex was originally chosen as an index of Cd toxicity. The ceiling dose used in this study (500 mg CdO/m^3) was expected to produce 250 mg/g Cd in the kidney cortex after 12-wk exposure, which should have caused slight renal damage (as reflected by increased proteinuria) (Friberg, 1974). However, the present finding, 10 mg/g Cd in the kidney, is far below the expected value. In addition, the proteinuria has not increased as a result of 70-day Cd exposure.

After reviewing Lal's Ph.D. dissertation (1976) and personal communication with K. Stemmer, lung and heart have been reconsidered as potential sites of early Cd toxicity after inhalation exposure. Lal (1976) exposed adult rats to 238 mg/m^3 CdO for 60 days and found emphysema and bronchiolar fibrosis, as well as myocardial degeneration. However, no kidney pathology was found.

Examination of lung and cardiac pathology is now in progess. Results of this examination will be taken into consideration concerning how much longer the exposure will continue and olfactory thresholds evaluated.

Publications - None to date

TEST FOR NEUROTOXINS USING CAENORHABDITIS ELEGANS

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Neurotoxic Disorders
1 R03 OH02095-01
06/01/85 - 05/31/87
$16,941 ($16,941 Cum)

Objectives

The overall aim of the project is to develop a screening test for neurotoxins using the nematode *Caenorhabditis elegans*. The test organisms are exposed to selected chemicals and changes in behavior are evaluated using computer tracking.

Methodology

A strain of *E. coli* is grown on experimental plates until a lawn is well formed and then 0.4 mL of an aqueous solution of the test chemical is added to the plates. The plates are desiccated for 24-hours at 50% relative humidity to allow for the excess water to evaporate. A number of three to four day-old *C. elegans* are placed on each plate. After 24-hours two types of evaluations are made: lethality and computer tracking to determine behavioral changes. Control cultures are tested concurrently with the neurotoxins.

With lethality tests, death is determined by the total lack of movement and/or response to probing with a needle. For behavioral studies, the worms are removed from the plates, washed, and placed on 1% agar. The worms are positioned under a video camera that is interfaced to a microcomputer. This arrangement allows for the simultaneous tracking of several hundred nematodes. The movements of the animals are analyzed to determine both the rate of movement and the number of changes in directions (reversals). In the future, responses to sensory stimulation will also be measured.

Progress & Accomplishments

Lethality studies have been completed for seven metals: Hg, Cu, Pb, Cd, Zn, Al, and Ni. For each metal a dose-response has been determined and the LC50 has been calculated using both probits and logits.

Computer tracking experiments have been conducted on Hg and to a lesser extent on Cu. The nematodes exposed to Hg show a biphasic response - at very low Hg concentrations there is hyperactivity that appears to peak at exposure concentrations of about 7 ppm Hg and as the concentration increases the activity falls to well below the controls. This peak activity Hg concentration is approximately 10% the nematode LC50 of 60 ppm Hg. The computer tracking for Cu has only shown decreased activity as the Cu concentration increases; however, very low concentrations of Cu have not yet been studied.

Significance

The lethality data has been compared to the LD50 data from mammalian studies and, except for one metal (Cd), there is a good correlation between the results. Although much more data is needed, it is believed that this test species shows much promise as a basic model for range-finding studies and initial lethality screening tests.

The computer tracking studies are in a preliminary state, but the hyperactivity observed with Hg supports our hypothesis that computer tracking of *C. elegans* can provide a rapid, inexpensive means of screening chemicals for neurotoxicity.

Publications - None to Date

THE EFFECTS OF IMPULSE NOISE ON THE AUDITORY SYSTEM

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Noise-Induced Hearing Loss
5 R01 OH01152-06
07/01/80 - 06/30/86
$123,163 ($588,879 Cum)

Objectives

The purpose of this research is to understand the relation between the parameters of impulse noise (peak pressure, duration, number, repetition rate, exposure duration, etc.) and the effects on hearing as assessed by anatomical, physiological, and psychophysical techniques in the chinchilla.

Methodology

Several mechanical or electrical-mechanical devices are used to produce realistic noise impacts. Hearing is tested, the animal is exposed to impulse/impact and its hearing is tested for the following forty days. Routine data collection consists of measurements of hearing sensitivity, auditory discrimination and cochlear histology. More detailed studies of certain experimental groups will include scanning E.M., as well as more discriminating psychoacoustic measures of hearing.

Progress & Accomplishments

This project has led to a number of new insights into the effects of exposure to impulse noise. We have provided data to support the hypothesized relation between the duration of an impulse and its hazard to hearing; we have shown (morphologically) that impulse noise can damage the cochlea by direct mechanical destruction. This mechanical damage is different than the cochlear damage following continous noise and is probably responsible for different audiological symptoms produced by impulse and continuous noise. Several years ago we showed that impulse and continuous noise can synergistically interact. We have documented that noise induces a number of the changes in the neural code leaving the ear and have related these changes to the symptoms of noise induced hearing loss. Recently, we have finished a large, parametric study of the validity of the Equal Energy Hypothesis.

Significance

The results have direct implications for noise standards. Heretofore, we have either neglected or underestimated the traumatic potential of impulse noise. Our research shows the peculiarities of impulse noise and suggests several situations where impulse noise increases the audiological hazard.

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FUNCTIONAL CORRELATES OF COCHLEAR INJURY

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Noise-Induced Hearing Loss
5 R01 OH02128-02
09/01/84 - 08/31/89
$108,034 ($217,315 Cum)

Objectives

The major goal of this project is to determine how the magnitude, pattern, and growth of hearing loss and structural damage are altered as the physical characteristics of the stimulus (frequency, intensity, duration, and scheduling of noise) are varied. Another goal is to determine the physiological correlates (as revealed by single-unit studies) of the behavioral changes and anatomical damage induced by exposure to noise. These behavioral, physicological, and anatomical studies share the long-term goal of identifying the relations between functional deficits and injury in the inner ear rather than specifying damage-risk criteria for human exposures. A final goal is evaluate presbycusis in a group of elderly chinchillas (8-19 years old) that have been raised in a sound-shielded environment and have never been exposed to noise.

In addition to these studies, acoustic measures (distortion products; spontaneous otoacoustic emissions) will be made from ear canals of noise-exposed chinchillas. These measures will provide physical indicators of cochlear integrity in the intact animal and information about nonlinear and active processes in the normal and damaged cochlea.

Methodology

Some of the experimental animals will be trained by food-reward, operant-conditioning techniques so that measures of auditory function (e.g. pure-tone thresholds, frequency and intensity difference limens, etc.) can be obtained before, during, and after their exposure to noise. Physiological studies will include thresholds and tuning curves of individual fibers, rate-level functions, and population studies in noise-exposed animals. Histological evaluation of the specimens will include detailed study of plastic-embedded flat preparations so that counts of missing sensory and supporting cells can be made and cytocochleograms prepared for all specimens. Selected regions of the organ of Corti will be sectioned and examined by light and electron microscopy and analyzed morphometrically. Acoustic measures are made with a small probe microphone assembly (Knowles) that is inserted into the ear canals of anesthetized chinchillas.

Progress & Accomplishments

1. Behavioral studies. We have continued evaluating hearing loss in chinchillas exposed to an octave band of noise (0.5 kHz, 95 dB SPL) for long durations. To date, we have found that ATS is stable for as long as 400 days; that is, the hearing loss measured during days 390-400 of exposure is no worse than the hearing loss measured in the first week of exposure. Despite this apparent stability of the auditory system during prolonged exposure, anatomical data indicate that the longer exposures are considerably more damaging to the cochlea than the short ones, and studies of PTS after exposure indicate a steady growth of PTS.

Another group of subjects has been exposed to the same noise on an interrupted schedule (15 min/hr for 400 days). The results confirm and extend our previous observation that under this schedule of exposure, chinchillas first develop hearing losses of 35-40 dB over the first 4-6 days of exposure, but as the exposure continues the hearing recovers in an exponential fashion, such that after 30-40 days of exposure the hearing losses are on the order of 10 dB.

2. Physiological studies. We have attempted to identify the locus of the recovery mechanism by means of a series of physiological studies. Auditory nerve AP thresholds and single unit tuning curves were determined in chinchilla ears exposed to the 0.5 kHz OBN at 95 dB SPL, 15 min/hr for 4 days (n=4) or 40 days (n=6). AP thresholds were elevated by 35-40 dB for the 4-day animals and 10-20 dB for the animals exposed for 40 days. Tips of tuning curves with low-frequency CF's were elevated in 4-day exposures, but nearly recovered in the 40-day exposures.

3. Acoustic measures. We have continued to examine the ears of noise-exposed chinchillas for spontaneous otoacoustic emissions (SOAE's). Since the behavioral experiments have utilized long duration exposures, we have exposed 6 untrained animals (12 ears) continuously to the 0.5 kHz OBN for as long as 108 days. Measures made before exposure did not reveal any cases of SOAE's, but after an exposure of 18 days one chinchilla did develop and SOAE (2.1 kHz, about 10-15 dB SPL). This emission is stable, and will be the subject of future studies.

Significance

The behavioral and anatomical studies on long-duration noise exposure will help elucidate relations between TTS, ATS, PTS and damage to the cochlea. The hypothesis that ATS predicts eventual PTS is extremely important and can only be addressed by studies such as these. Our finding that the ear can recover 30 dB of sensitivity during intermittent exposure is of practical and theoretical interest. Physiologists must now consider an active process within the cochlea that can restore as much as 30 dB of sensitivity to a damaged ear. Practically, these findings suggest that workers exposed on various intermittent schedules may not exhibit significant threshold shifts even though the noise exposure is damaging the cochlea and may eventually lead to a PTS. Finally, our studies of SOAE's in noise-exposed animals provide a unique opportunity to study the relations between experimentally-induced SOAE's and cochlear histopathology. These studies will lead to a better understanding not only to emissions themselves, but also of active cochlear biomechanics.

Publications - Abstracts available

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INDUSTRIAL NOISE: POTENTIATING INTERACTIONS

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Noise-Induced Hearing Loss
7 R01 OH02317-01*
08/01/84 - 07/31/87
$106,450 ($198,226 Cum)

*Previously supported under Grant Number R01 OH01518

Objectives

The objective of this grant is to systematically examine the synergistic effects of noise exposure and vibration on hearing. Two different types of noise exposure [three band-limited noises of equal energy and five impulsive noises with different levels and types (A or B duration)] and two examples of whole-body vibration at different frequency and magnitude are being combined to assess the factors that influence the interaction between vibration and various noise exposure paradigms.

Methodology

A series of 10 control groups and 17 experimental groups is being exposed to continuous or impulsive noise, vibration or a combination of noises or vibration and noise. The exposures utilize an experimental paradigm which produces an asymptotic threshold shift. Hearing thresholds and masked thresholds (i.e., tuning curves) are obtained before exposure and over a thirty-day post-exposure period using auditory evoked potentials. The final format of the data includes a comprehensive description of the exposure, a profile of the animals hearing ability before and after the treatment and a detailed, quantitative morphological analysis of the cochlea. The morphological analysis relies primarily upon suface preparations of the organ of Corti and the vascular tissues of the lateral wall of the cochlea and includes scanning and transmission electron microscopy of interesting or unusual pathologies.

Progress & Accomplishments

Eight control groups (noise) and four experimental groups (continuous and impulsive noise) have been completed. It is premature to discuss the results of specific exposures at this time. However, the data are orderly and, in general, there is an increased effect in the combination exposure groups. The anatomy, acoustics and evoked potential laboratories have been placed under computer control.

A computer-controlled morphometric system and associated software has been developed for use in the analysis and evaluation of the cochlear sensory epithelium and vascular tissue. The system is capable of digitizing a microscopic image and evaluating a variety of predetermined variables such as sensory cell populations, supporting cell, vascular variables (e.g., red blood cell density, capillary volume, pigment density, etc.).

Computerization of the acoustics laboratory allows not only the efficient collection of physical data on exposure conditions, but allows the generation of impact or continuous noises with very specific physical characteristics such as amplitude and phase spectra, various energy loadings, etc. This capacity allows the design of exposures which are tailored to specifically evaluate current strategies for damage risk criteria (e.g., equal energy hypothesis, the use of various weightings, etc.).

The evoked response laboratory has been brought under computer control. Software developed in this laboratory allows for the collection of evoked potentials to an intensity series across several frequencies. Alternatively, new software employs statistical criteria for the detection of a "response" within the evoked potential, thereby allowing for an objective determination of threshold (absolute or masked) and removing possible subjective factors such as tester bias from influencing the final determination of subject threshold.

Significance

Synergistic interactions among various ototraumatic agents have been shown to be of importance in determining the hearing loss in a number of industrial situations. The interactions between continuous and impulse noise and vibration and noise (both continuous and impulsive) are being studied using an animal model. The rationale for these studies is that continuous noise rarely exists as a sole hazard to hearing in most industrial and military work environments. Thus, exposure criteria which are based upon data obtained from experiments involving only the use of continuous noise may not be protective.

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NOISE-INDUCED HEARING LOSS AND HIGH BLOOD PRESSURE

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Noise-Induced Hearing Loss
5 K01 OH00042-02
09/28/84 - 08/31/87
$31,320 ($63,720 Cum)

Objectives

The goals of our present study are: 1) further validation of our previous noise and blood pressure study (Talbott, Amer Jour Epid 121:4, 501-514, 1985) by its replication in a group of men 55-65 who have long histories of occupational noise exposure, 2) to carry out further more detailed characterization of the audiometric profile of these occupationally noise-exposed men, and 3) administration of the revised form of the Hearing Performance Inventory as developed and tested by Lamb et. al. (Journal of Ear and Hearing, 1983). This is being used to assess the communicative difficulty of noise-exposed individuals in a variety of everyday listening situations. In addition, an index of social support is being administered as that was a variable found to correlate with B/P in our previous study.

Methodology

A cross-sectional design was selected in order to test the hypothesis and study the relationships already described. The men are retired metal assembly workers aged 55-65. There are 600 active members in the retirement organization. The criteria for eligibility are that the individuals be white male hourly workers between the age of 55-65 and must have 20 or more years of exposure on the job. The average ambient noise level in this plant is ≥ 89 dBA. We have chosen this group because: 1) They represent the most exposed segment of the plant population, 2) the previous study indicated a strong effect for severe noise induced hearing loss and blood pressure in this group, 3) we hope for a high response rate (to rule out self-selection bias) and this group is more accessible. Union records were used to obtain the worker's name, address, department where he worked and seniority date.

In order to assure sufficient group size for stratified cells and noise-induced hearing loss comparisons, a total sample of 250 men age 55-65 should be sufficient.

The clinical exam includes measures of height, weight, pulse and blood pressure, as well as an indepth medical and personal habits history including alcohol consumption and smoking patterns. There is also an occupational history, military and noisy hobby questionnaire and lifestyle interference inventory. This exam is being administered at the Union Hall in the morning.

Because large variations in blood pressure have been shown to exist within a given subject, it is difficult to detect differences between groups and may lead to misclassification of individuals. Therefore, multiple blood pressure measurements using standard procedures have been implemented in this investigation. Blood pressure is determined three times within five minutes after a ten minute rest period and then repeated by a second staff fifteen minutes later.

Standardized audiometric testing procedures are being used and the technician is supervised by a Ph.D. audiologist who possesses a certificate of clinical competence in audiology from the American Speech and Hearing

Association. Other audiometric tests to be included are: 1) pure tone air conduction, 2) bone conduction, 3) speech reception threshold, 4) speech discrimination scores in quiet and in competing noise, and HPI.

Progress & Accomplishments

After a period of recruitment of personnel into the study, we have trained 2 nurse epidemiologists in blood pressure measurement, and an audiometric technician has been recruited to work on the study. A list of eligible retirees has been also obtained and survey instruments selected. The questionnaires and methods were piloted during the period of December, January, and February. It was during this time that we also reviewed various questionnaires and hearing assessment inventories to determine which would best suit the needs of the men in this particular study. We began screening in March 1985. To date we have attempted contact of 120 individuals via letter to request participation in the study and indicating that we would be calling them to set up a clinic date in the near future. Of these, 16 were found to be ineligible because of age or life threating illness. An additional 19 either unpublished numbers or return-to-sender addresses which must be traced. We will be sending another letter to the individuals with unpublished numbers requesting that they call our office. There have been only 8 refusals thus far. A total of 80 individuals have completed their screening. Coding of the data for statistical compilation has not begun and therefore we do not have any pertinent analysis at this time.

Significance

The results of our previous study suggest that severe noise-induced hearing loss and blood pressure levels are associated and both may be due to long-term noise exposure among susceptible individuals. An alternate hypothesis may be that in the older worker, noise exposure in addition to the aging process may lead to poor speech discrimination, discomfort to loud sounds, etc., such that it may result in reduced social interaction and interference in lifestyle and increased stress which may affect B/P levels. This study is designed to address this question.

Publications - None to date

ACOUSTIC STAPEDIUS REFLEX AND TEMPORARY THRESHOLD SHIFT

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Noise-Induced Hearing Loss
5 R03 OH01718-02
07/01/83 - 12/31/85
$22,500 ($42,330 Cum)

Objectives

Clinical and experimental observations indicate that there are individuals uniquely susceptible to noise-induced shifts in auditory sensitivity. Experimental induced temporary hearing loss varies widely in subjects exposed to exactly the same noise in level and spectral composition. The acoustic reflex (AR) provides a protective function that also varies from individual to individual. Thus, the AR may be one of the many factors that relate to the development of temporary threshold shift (TTS). The purpose of this project was to determine if dynamic properties of the AR (threshold, latency, magnitude and decay) relate to TTS produced by two-hour exposures to broadband noise and two different octave-band noise (0.5 and 3.0 kHz). Additionally, TTS was correlated to case history data, pre-exposure behavioral thresholds and ear canal volume in linear and multiple regression models.

Methodology

Two separate, two-hour noise exposure studies were completed to determine which, if any, of the properties of the AR correlate to TTS produced be different noise stimuli. Careful measurements of the AR were obtained to different eliciting stimuli prior to each exposure. Behavioral thresholds from 0.25 through 8.0 kHz were obtained from trained subjects before and after exposure. Acoustic immittance outputs were digitized and subjected to algorithms designed to evaluate AR threshold, latency magnitude and decay. Ear canal volume and case history information (age, sex, eye color, use of tobacco, etc.) were obtained on all subjects. Behavioral hearing sensitivity (method of adjustment) was established with the aid of a computer.

The first study established correlations among these measurements and TTS produced in 37 subjects exposed to broadband noise. The second study explored correlations among AR dynamics and TTS is 30 subjects who were exposed on different days to both 0.5 and 3.0 kHz octave-band noise.

Progress & Accomplishments

To accomplish the objectives of this project, studies were designed to identify correlates between AR activity and TTS produced by exposures to broadband noise and octave band noises centered at 0.5 and 3.0 kHz. All data has been collected and analyzed and efforts are under way to write the final report and complete manuscripts for publication.

Acoustic reflex magnitude and decay elicited with broadband noise correlated to many expressions of TTS. The greater the magnitude of response ($r= -.40$) and the less the amount of decay ($r= .33$), the smaller the TTS. Test results indicated a relationship between ear canal volume and the frequency at which maximum TTS occurred. Subjects with greater TTS at 3.0 kHz had ear canal volumes that were significantly greater than subjects with greatest TTS at 6.0 kHz. Acoustic reflex threshold and latency and case history information did not correlate with TTS. Multiple regression modeling that included all significantly related variables improved TTS predictability over simple linear regression modeling. The second series of studies examined the relation of dynamic properties of the AR to the TTS that developed in thirty subjects following two separate, two-hour octave band noise exposures. Pre-exposure behavioral thresholds and several AR parameters were found to be significantly correlated to the TTS that resulted from both exposures. Multiple regression modeling showed AR adaptation, onset latency and magnitude to be only slightly predictive of the TTS that resulted from the low-frequency exposure.

Overall, results from these studies suggest that AR is one factor in the development of TTS following two-hour exposures. Only a slightly predictive relationship exists, however. The strongest single predictive property of the AR is an expression of its sustained activity.

Significance

The development of a test battery to identify those individuals susceptible to the hazards of noise exposure has been sought for decades. The results suggest that the AR influences the development of TTS, however is only slightly predictive. Acoustic reflex adaptation (decay) accounts for less than 30% of the variability in TTS. Thus, measurement of the AR may have predictive strength as part of a larger test battery designed to include other parameters. Additionally, ear canal volume is a factor influencing the pattern of TTS. Results suggest that persons with larger ear canal volumes will suffer greater TTS in the lower frequencies than persons with larger volumes.

Publications - Abstract(s) available

HEARING PROTECTION: SELECTION AND COMMUNICATION ISSUES

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Noise-Induced Hearing Loss
5 R03 OH01829-02
09/28/84 - 08/31/86
$19,500 ($40,308 Cum)

Objectives

This two-year research program addresses issues which have direct bearing on the selection and utility of hearing protection devices (HPDs) for attenuation of industrial noise. This aim of the first year's research is to determine experimentally the differential effectiveness of various HPDs, including earmuffs, earplugs, and ear canal caps, in attenuating sound reaching the ear for different frequencies. Different types of HPD application-instructions given to users may also affect the amount of noise protection afforded by HPDs and therefore this variable is also under study. In general, achieved earmuff attenuation would be expected to be less influenced by instruction than that of earplugs, which are usually more difficult to don. During the second year of the program, the research objective is to compare, under controlled conditions, the levels of interference produced by different HPDs on human speech communication in different intensities and spectra of recorded industrial noise.

Methodology

In the HPD noise attenuation study, five universal-fit earplugs, four earmuffs, and two ear canal caps were assessed. The REAT (real-ear attenuation at at threshold) method was employed to determine the attenuation afforded by each HPD using each of 5 levels of instruction. A free-field loudspeaker system was used to present hearing test tones at 125, 250, 500, 1000, 2000, 4000 and 8000 Hz to subjects with normal hearing seated in a near-anechoic chamber. Therefore, for each HPD, a frequency-specific "profile" of attentuation capability was determined for each instruction condition. In parallel with this laboratory effort, a field survey of numerous typical industrial machine noise emissions was performed, resulting in 1/3-octave noise spectrum plots for each emission. By comparing the attenuation spectra obtained in the laboratory with the noise spectra obtained in the workplace, optimal matching of HPD and noise source can then be accomplished.

The methodology for the second year's experiment concerning HPD effects on speech intelligibility is currently being finalized. The experiment will utilize the actual HPDs and recordings of industrial noise sources obtained during the first year, as originally planned. Subjects will listen to speech stimuli, and respond accordingly, in the presence of the different masking noise spectra. Both the spectra and the S/N ratio will be varied and the actual industrial noise recordings will be used for the masking spectra. Subject will perform the speech intelligibility tests both with occluded ears (HPD on) and with unoccluded ears to determine the effect of each HPD on speech intelligibility in each spectrum of noise. Dependent measures of correctly identified speech phonemes and response latencies will be obtained. Experimental protocol will include the use of a semi-reverberant audiometric chamber and placement of HPDs by an experimenter trained in audiology. Individual differences in baseline hearing levels across sound frequencies will be balanced across experimental conditions.

Progress & Accomplishments

All experimental tasks, associated analyses, and reports for the HPD attenuation study and the industrial noise survey have been completed. The frequency-specific noise attenuation capabilities for each HPD, plotted as dB as a function of frequency in Hz, are now available. More importantly, HPDs were found to differ widely in the level of noise protection they provided based on the level of user application-instruction given. Briefly, earplug attenuation was found to be much more susceptible to differences in instruction than was earmuff attenuation. Certain detailed, systematic insertion-instructions resulted in as much as threefold increase in noise protection in dB over no or minimal instructions for earplugs. Earmuffs were consistently more resistant to differences in instructions than earplugs. For earmuffs, in general, most all types of instructions resulted in significantly higher noise attenuation than no instruction at all. Other differences in attenuation as a function of subject gender and in HPD comfort/user preference were found; these are discussed in detail in the publications listed below.

The industrial noise survey results (recordings) will be used in the speech intelligibility study for which data will be collected in Spring 1986, as originally scheduled. Instrumentation preparation and subject screening for this study are currently underway.

Significance

Because HPDs differ widely in their spectra of attenuation and noises in their spectra of power, it is essential that HPD spectral attenuation data be obtained and matched to noise spectral data to find the optimal HPD for the given noise problem. This is evidenced by the data obtained in studies performed in this research to date. Furthermore, it has been demonstrated that user application-instruction plays an important role in achieving reasonable noise protection with an HPD. Workers who do not receive proper instruction in fitting and wearing their HPDs may believe they are well-protected, but really may not be. Also, the level of instruction available has direct bearing on an industrial safety officer's selection of HPDs for his/her workforce, because certain types of HPDs are less susceptible to instruction than others.

The results of the second year's experiment on speech intelligibility effects of HPDs will help provide further basis for HPD selection, when a particular type of noise spectra is involved and when speech communication must be maintained. Furthermore, the data will hopefully aid in dispelling the notion that HPDs hinder speech reception in high noise environments.

Publications

Epps BW, Casali JG: Hearing protection device comfort and user preference: An investigation and evaluation methodology. Proceedings of the 1985 Human Factors Society 29th Annual Conference, Baltimore, Maryland, September 29-October 3, 1985, 814-818.

Casali JG, Lam ST, Epps BW: Influence of insertion/donning instruction on frequency-specific sound attenuation achieved with earplugs, ear canal caps, and earmuffs with implications for industrial noise application. Blacksburg, Virginia: Virginia Polytechnic Institute and State University, Department of Industrial Engineering and Operations Research, Technical Report Number 8506, August, 1985.

AUDITORY TEMPORAL ACUITY FOLLOWING NOISE EXPOSURE

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Noise-Induced Hearing Loss
1 R03 OH02141-01
09/27/85 - 08/31/87
$19,484 ($19,484 Cum)

Objectives

The purpose of the study is to describe subtle, temporary changes in human temporal acuity brought about by listening to moderately intense noise for a brief period of time. The effect of signal frequency, signal level, and recovery time on gap detection threshold will be examined following the noise exposures.

Methodology

Octave-band noises with a brief temporal gap will be used as the experimental stimuli. Off-frequency spectral cues will be minimized with a broad-band masker. An adaptive two-interval forced-choice psychophysical paradigm will be used to measure gap detection threshold (GDT). The GDT's will be obtained both before and after a 5-minute, octave-band noise exposure. Simulus generation and control of the experiments will be accomplished with a laboratory microcomputer.

Progress & Accomplishments

To date, the accomplishments related to the project include: (1) selection and purchase of a microcomputer and related laboratory apparatus, (2) equipment set-up, and (3) development of computer software to generate the stimuli and control the experiments.

Significance

The results may provide the basis for developing a more sensitive tool for evaluating the effects of noise on hearing. Information from this project may also help us understand the temporal processing capabilities of some hearing-impaired individuals.

Publications - None to date

INSTRUMENT FOR WORKSITE EVALUATION OF HEARING PROTECTION

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Noise-Induced Hearing Loss
1 R43 OH02136-01
09/28/84 - 07/31/85
$50,000 ($50,000 Cum)

Objectives

To prevent noise-induced hearing loss through development of an instrument for worksite measurement of true effectiveness of insert type hearing protection devices (HPD's) "as worn".

Methodology

1. To develop and program a subject-interactive laboratory instrument, interfaced with a micro-computer, which will provide diverse auditory test signals and which will collect and analyze subject response data.
2. Using this computerized laboratory instrument, program and evaluate in the laboratory a number of suprathreshold techniques of measuring HPD attenuation.
3. Build a prototype field test unit incorporating the most saftisfactory test method determined with the laboratory instrumentation.
4. Complete laboratory and initial field testing of the protype portable field test unit.

Progress & Accomplishments

The laboratory instrumentation has been designed, a prototype constructed, and programming for the laboratory testing has been written. The prototype instrumentation and programmming is currently being evaluated. This instrumentation is a five-channel microprocessor-based auditory signal generator capable of delivering signals to circumaural earphones and/or external loudspeakers. The output from each channel, in terms of signal type, timing (rise & decay, on-off times and pulse train characteristics) and routing, is controlled by software developed for the micro-computer. The menu-driven program is subject-interactive via a hand-held response device that modulates the signal outputs. All signals and responses are recorded by the computer both on-line and later analysis.

Any number of suprathreshold of threshold test routines, along with aural or ambient masking strategies, can be operator introduced at the computer keyboard. Visual response feedback is proved to the subject by LED's on the hand-held response device as a function of the test in use.

Forty-five test subjects have been screened and accepted for the laboratory test phase of this study. Acceptance was based on a negative history of otologic disease, a normal otologic examination by an ear specialist and a normal puretone auidogram. These subjects are awaiting further participation in the study pending final evaluation of the test instrumentation and programming.

A concurrent objective has been to determine, on the basis of previous research in the field, what single auditory test frequency (or combination of frequencies) should be used to most accurately predict overall attenuation of insert type HPD's. This single frequency, or combination of frequencies, will be used in our suprathreshold tests of HPD attenuation.

Test frequency determination was based on extensive correlation of previously published octave-band measurements of environmental noise with insert-type HPD noise attenuation values. A computer program was developed to manipulate the more than 700,000 individual calculations. This analysis has been completed and the test frequency selected. A detailed report of this correlation will soon be sumbitted for publication.

Significance

Field investigations by the National Institute for Occupational Safety and Health have indicated that 50% of the workers (wearing HPD's) received less than half of the potential protection demonstrated in laboratory testing. Recent articles and studies have addressed the inaccuracies of the current practice of "assumed attenuation data" based on laboratory testing of HPD's and they point out the need for an adequate method for measuring the "field attenuation of hearing protectors" in the real world. Our device under development is designed to rapidly and reliable assess the effective attenuation of insert-type HPD's at the worksite. Its design allows an unsophisticated operator to apply the test to an unsophisticated subject (i.e. workers wearing HPD's). Because testing is done at above-threshold levels, results are impervious to ambient noise.

Potential purchasers in private industry number approximately 60,000 as well as an estimated 5,000 facilities in the military, transportation and service industries.

Publications - None to date

BIOASSAY FOR DIOXIN: WORK PLACE RELATED CUTANEOUS HAZARD

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Dermatologic Conditions
5 R01 OH01533-03
11/01/83 - 10/31/85
$41,759 ($85,814 Cum)

Objectives

The overall goals of this project are to evaluate two in vitro markers of dioxin induced biological activity as an assay(s) for dioxinlike chemicals and the ultimate application of such an assay(s) to the detection of these chemicals in biological samples relevant to occupational settings. The two markers are 1) the induction of an in vitro keratinization response in XB epithelial cells cocultured with irradiated fibroblast feeder cells and 2) the induction of a proliferative and morphologic alteration in XBF epithelial cells, which were derived from the XB line, also cocultured with irradiated fibroblast feeder cells. The most toxin dioxin isomer, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was used as the dioxin prototype.

Methodology

These studies are based on in vitro alterations in epithelial cell culture by TCDD. Briefly, the semiquantitative determination of this effect was determined as follows:

Epithelial target cells were suspended by trypsinization and seeded into 24-well plates (16-mm diameter wells, 5 x 10^4 cells/ml per well) with irradiated 3T3 cells (5 x 10^5/ml per well) in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum. After overnight incubation (37°C, 5% CO_2, humidified) the cultures were refed with a series of 10-fold dilutions of a stock solutrion of test chemical or sample extract in Me_2SO concentration was 0.1%. After 14 days the culture was assessed for flat-cell induction, washed with phosphate-buffered saline (PBS), fixed with Formalin in PBS, and stained with either Giemsa or 1% rhodamine B in water.

The cultures were evaluated for the flat-cell effect by phase-microscopic assessment and confirmation that the tested cultures had grown to form a confluent monolayer of morphologically flat cells as compared to the high density, control cultures comprised of multilayered fusiform cells.

Progress & Accomplishments

Initial studies were done to determine optimal conditions and evaluate each system. These studies involved the effects of frozen storage, variation of culture conditions e.g. - serum concentration, feeder cell concentration and effect of serum lot number. Experiments were also done to establish new, more stable and responsive clones from the parent lines. The results of these studies indicated that the XB cell keratinization system, which was found earlier to lose its keratinization induction phenotype with cell passage, was unable to generate subclones in which this phenotype was stable. These studies indicated that low passage frozen XB stocks could be used continued studies of this phenotype, but that the use of this system as an assay for dioxinlike activity could be limited. These studies also demonstrated the application of the cell keratinization system to the

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analysis of soot samples contaminated with polychlorinated dibenzofurans. Based on these findings, the majority of the remaining work was performed on the XBF line and the dioxin induced morphological alterations from high density fusiform to a low density, flat epithelial morphology referred to as the flat-cell effect (FCE).

Studies were done to define the FCE which was shown to be the result of a reversible inhibition of post-confluent proliferation. Both treated and control XBF/3T3 cultures grew at equal rates until confluency was reached at which time the TCDD treated cultures became quiescent while the control cultures grew to a higher saturation density. The nondividing treated cultures were shown to be viable, and upon resuspension by routine trypsinization, were capable of further growth and of again exhibiting the FCE upon exposure to TCDD.

Validation studies were performed to determine the specificity and sensitivity of the FCE to various chemicals. Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurnas (PCDFs), polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), and pesticides (24 compounds in total) were tested for their ability to induce the FCE. The results indicated that these changes are relatively specific for PCDDs and PCDFs. TCDD was the most potent congener tested, capable of inducing the effects at a concentration as low as 10^{-11} M. The activities of other tested PCDDs and PCDFs ranged from 10^{-1} to 10^{-3} of TCDD activity. The PCBs, PAHs, and pesticides had lower activities ranging from 10^{-3} to 10^{-6} that of TCDD.

Ten extracts of soot containing PCDFs from an office building involved in a transformer fire were tested for FCE inducing ability and the results were compared to a high resolution gas chromatographic/mass spectrometric analysis (GC/MS) for total PCDFs in the same samples. This comparison showed a good correlation, suggesting that the XBF/3T3 system has potential use as a semiquantitative assay for dioxinlike activity.

Preliminary experiments using the FCE, done under the optimal conditions as determined by the above results, have been performed to determine the effect of exposure of TCDD to serum on the efficacy of the flat-cell assay. These studies, using fetal bovein serum, indicate that TCDD-serum interactions do not interfere with the sensitivity of the assay.

Current investigations concern the application of the flat-cell assay for dioxinlike activity to biological samples. These studies will focus on the detection of this activity in blood and involve in vitro and in vivo investigations.

Significance

These studies demonstrate that an in vitro assay for dioxinlike activity, based on TCDD induced alterations directly related to epithelial differentiation, has been developed. Chloracne is one of the most common effects of TCDD exposure in humans and is considered to be the result of induced differentiation. The described bioassay system therefore has potential usefulness in screening biological samples of various types (blood, urine, milk) from workers in occupational settings which carry a high risk for dioxin exposure. This will allow detection of the exposure incident and a semiquantitative evaluation of the degree of exposure. Other uses include the detection of dioxinlike activity of active dioxin analogues in the workplace and evaluation of structurally similar compounds.

Publications

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Kaminsky LS, DeCaprio AP, Gierthy JF, Silkworth JB, Tumasonis C: The role of environmental matrices and experimental vehicles in chlorinated dibenzodioxin and dibenzofuran toxicity. Chemosphere 14(6/7):685-695, 1985.

MECHANISMS OF OCCUPATIONAL LEUKODERMA

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Dermatologic Conditions
5 R01 OH01556-03
09/29/82 - 03/31/86
$55,233 ($149,944 Cum)

Objectives

Occupational depigmentation from phenolic and catecholic skin depigmenters (DP) involves a direct attack on melanocytes. The susceptibility of melanocytes to DP may directly stem from physical and chemical interactions of DP with pigment melanin and/or tyrosinase. Binding of DP to melanin would "select" the pigment as a target for chemical attack. Subsequent redox reactions may involve either electron transfer from DP to melanin or "autoxidation" of DP by O_2 (probably catalyzed by tyrosinase). These reactions could undermine the ability of melanin to protect the cell from cytotoxic agents and could result in diffusible reactive species capable of causing melanocyte death. To test this hypothesis we propose to (1) measure the binding and redox interactions of selected phenolic and catecholic compounds of widely varying potency with model synthetic melanins, melanin-tyrosinase complexes and with melanoproteins extracted from C-57-BL-6 black mice. The kinetics of the above-mentioned redox reactions will be characterized. Experimental conditions will be chosen which maximize a given component reaction while minimizing the others. This will allow an approximate separation of the overall interaction into binding, "autoxidation" and electron transfer components. We will use these results as a basis to dissect the total DP-melanosome interaction into simpler components. (2) induce hair depigmentation by local subcutaneous or intradermal injection of DP into black mice. The relative potencies of the DP's will be estimated visually and by a turbidimetric analysis of hair melanin in alkaline-sulfide medium. (3) Seek possible correlation between DP potency and the behavior of one or more component interactions.

Methodology

We have expanded the scope of the previous studies to include (a) synthetic DOPA-Melanin prepared by incubation of L-DOPA with tyrosinase, (b) melanoprotein extracted from C57-BL (black) mouse hair (connection to "Animal Studies"), (c) melanin-tyrosinase complexes (probably more "relevant" to in vivo situation) and (d) extracted bovine eye-melanin (a relatively easy-to-obtain melanoprotein).

The following compounds are studied: (a) t-butylacatechol (tBC), (b) 4-methylcatechol (MCAT) (substituted catechols); (c) hydroquinone (HQ); (d) catechol; (CAT) (e) 4-t-butylphenol tBP (a substituted phenol); (f) DOPA: (g) 3,4 dihydroxyphenylacetic acid (DHPA), (substituted catechol).

We assess the ability of each DP to act as a substrate for tyrosinase. The oxygen uptake and spectrometric data are analyzed for each DP according to the two schemes. The qualitative and quantitative results will be compared with each other and will data on the non-enzymatic oxidation.

To assess the effect of melanin, 0-100 ug will be incubated with DP - O_2 - tyrosinase systems. Spectroscopic changes and O_2 uptake will be monitored as before. Melanin interaction will be assessed by one or more of the following: (a) change in the time-course of DP oxidation and/or product formation (b) change in V_o, (c) change in Km or Vm, and (d) changes in product distribution.

Electron transfer kinetics from DP to test melanin will be determined by measurement of the rate of reduction of potassium ferricyanide, and electron acceptor. If melanin couples electron transfer from DP to ferricyanide, then reciprocal plots of initial velocity vs [DP] will be linear. From the slope and intercept, one can obtain k', a rate parameter and K_B, the binding constant for DP to melanin. These parameters are characteristic for given DP and melanin preparations.

Animal Studies will be carried out with C57-BL mice. CP-1 mice will be used as controls. Hair will be removed from the dorsal surface of the black and the white mice by clipping. The clipped hair will be analyzed for melanin content. Black mice will be injected subcutaneously five days per week for 4 weeks with 0.01-1% of each DP in 0.1 ml physiological saline on the right side of the back. On the left side, 0.1 ml of physiological saline alone will be injected. White mice will be treated similarly. Readings of skin color and appearance, hair regrowth pattern, color and density will be made for 4-6 weeks. When sufficient hair has grown back (3-4 weeks), samples from around the areas of injection of DP and saline, as well as from a non-injected area will be analyzed for melanin content in both black and white mice. Visual and quanitative observations will be recorded.

Progress & Accomplishments

We have studied the thermodynamic and kinetic interaction of 10 catechols and phenols with synthetic L-DOPA-melanin. Using ferricyanide as electron acceptor, we have demonstrated that DP-melanin binding can lead to alterations in electron transfer kinetics, and that the magnitude of such alterations may be roughly correlated with depigmenting potency.

Spectroscopic analysis of reaction mixtures of DP oxidized by ferricyanide and by periodate in the presence or absence of L-DOPA melanin have provided a preliminary description as to oxidation product distributions. These results indicate that i) melanin does not change the product distribution or extent of reaction, and ii) the quinones of those catechols which are potent DPs are stable in solution, whereas those which are poor depigmenters are not.

Preliminary studies on the oxidation of DP by molecular O_2 (as followed by fluorescence measurement) in the presence and absence of melanin indicate that (i) such reactions are slow compared to FC and tyrosinase-catalyzed DP + O_2 reactions and (ii) melanin does not appear to affect the course of reaction. These results indicate that non-catalyzed DP + O_2 reactions can be neglected, in the first approximation.

We have experimentally (and reproducibly) produced depigmentation with t-butylcatechol, (tBC), 4-methyl-t-butycatechol (MCAT), catechol (CAT), hydroquinone (HQ), and monomethyl ether of hydroquinone (MMEH) at several concentrations. We have alos demonstrated that L-DOPA and 3,4 dihydroxyphenylacetic acid (DHPA) are non-depigmenters under the same conditions. We have documented characteristics distribution patterns, have obtained histological data from skin biopsies, and have obtained preliminary data on the kinetics of reversibility, as judged by the time course of pigment reappearance.

Preliminary results with mushroom tyrosinase indicate that DOPA, tBC, DHPA, MMEH, and tBP are substrates. Dopa-Melanin markedly accelerates the reactions in the case of MMEH nd tBP, to form 1,2 quinones. The mechanism of this interaction appears to involve the ability of melanin to (a) act as a "cofactor", analogous to DOPA, and (b) facilitate oxidation via electron transfer.

Significance

These results show the considerable influence of melanin on reactions which may lead to cytotoxicity. These results will help in filling the gaps necessary for realizing the following long-term goals: (a) early detection and prediction of environmental depigmenters, (b) attainment of a reliable regimen for effecting controlled depigmentation, (c) effective use of the appropriate DP's as chemotherapeutic agents in malignant melanoma, and (d) determination of mechanistic similarities and differences between occupational depigmentation and mechanistic similarities and differences between occupational depigmentation and vitiligo.

Publications - Abstract(s) available

EPIDERMAL LANGERHANS CELLS: IN VITRO STUDIES

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Dermatologic Conditions
5 R01 OH01955-02
09/01/83 - 08/31/85
$33,039 ($70,274 Cum)

Objectives

Epidermal Langerhans cells (LC) express many properties characteristic of macrophages and play a pivotal role in the development and elicitation of allergic contact hypersensitivity, and are possibly involved in immune surveillance of cutaneous tumors. Specific objectives:

1. Identification of factors (interferons, glucocorticosteroids) affecting the structure (cell surface antigens and receptors) and biosynthetic function of LC.
2. Quantitation of LC in skin samples from normal and immunodeficient individuals.
3. Establishment of a continuous cell line of human epidermal cells retaining LC characteristics.

Methodology

Reproducible immunologic and immunomicroscopic techniques for the identification of LC, the ability to isolate and maintain viable LC, immunologic techniques for enrichment in and depletion of LC and somatic cell hybridization techniques have been employed by our laboratory to complete a series of experiments.

Progress & Accomplishments - Refer to publications

Significance

Having elucidated the opposing roles of interferons and glucosteroids on LC expression of DR antigens may lead to a rational for their judicious use in specific immunoenhanced and deficient diseases including those studied in objective number 2. Establishment of a continuous cell line of human epidermal cells suggests a constant source of epidermal cells and has allowed for the systematic study of agents modifying these cells as a model for epidermal cells in vivo. In addition, in light of our success using somatic cell hybridization, efforts are continuing to isolate clones retaining LC characteristics.

Insights into possible therapeutic regimens to prevent or suppress contact hypersensitivity manifested as allergic contact dermatitis, may be gained with the identification of LC products and modualtors, The establishment of a line of human LC would facilitate the study of their biosynthetic properties.

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A PERSONAL DOSIMETER FOR EXPOSURE TO ULTRAVIOLET LIGHT

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Dermatologic Conditions
5 R01 OH02021-02
02/01/84 - 01/30/87
$84,329 ($188,945 Cum)

Objectives

There are uniformly ignored federal standards for near (315-400 nm) and far (200-315 nm) UV radiation. The standards are written with a flat response curve in the near UV and a steep curve in the far UV. The purpose of this study is to provide simple, low-cost, easily-analyzed, and interference-free dosimeters which meet the requirements of the standard and would be acceptable to the individual wearer for occupational, recreational, or therapeutic exposure to potentially carcinogenic ultraviolet light. The major hypothesis is that photochemistry of some appropriately chosen molecules could provide the required dosimeters by measuring the ratio of product to reactant.

Methodology

For the 200-315 nm region, a tube containing acetaldehyde vapor in air is to be worn in an exposed place. The methane produced is then analyzed after the end of the shift or after suspected exposure, using a gas chromatographic technique, Dilute NO_2 is used for the 315-400 nm range, and analyzed by chemiluminescence for the NO_2 removal.

The far UV photolysis is carried out in quartz tubes whereas the near UV photolysis uses pyrex tubing, thus eliminating any far UV phontons. The molecules chosen provide a very close match to the required spectral shape for the radiation exposure standards, particularly in terms of the sharp cutoff at long wavelengths. This eliminates any possible interference from abundant long wavelength photons.

Progress & Accomplishments

We have demonstrated that NO_2 photolysis and analysis of the NO_2 removal can be used as a dosimeter for near UV radiation and that acetaldehyde vapor can be used for the far UV. We have, however, encountered unexpected containment difficulties in that neither gas could be maintained in their containers for a long enough period for useful dosimetry. Two approaches to eliminate this difficulty are under test. The first is to use acetone rather than acetaldehyde. The former is a much more stable molecule. The second approach is to use a water solution of one of the relevant molecules (acetone or acetaldehyde for far UV) since much larger absolute numbers of molecules can easily be placed in the same container.

Significance

The original hypothesis has been proven to be correct. When the unexpected containment problem is solved, a very useful protective device will be the outcome.

Publications - None to date

BEST COPY AVAILABLE

CUTANEOUS EXPOSURE: PREDICTIVE PATHWAYS

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University of California	09/28/84 - 08/31/87
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Objectives

The ultimate goal of this research is to attain the ability to predict the detrimental toxic effects of hazardous chemical exposure via the skin. Specifically, our objective is the quantitative estimation of the toxicokinetics of occupationally-encountered molecules absorbed across human skin _in vivo_. The aims may be summarized as:

a. Establish a literature database on percutaneous absorption (PA).
b. Explore pathways by which a prediction of PA kinetics may be made.
c. Develop, refine and apply new predictive models for cutaneous toxicity.
d. Assess the value of different pathways in estimating multiple exposure risks.
e. Review critically _in vivo_ animal models and _in vitro_ (excised skin) techniques for measuring skin penetration and to determine whether straightforward relationships exist between these systems and the human _in vivo_ situation.

The long-term focus of the project is to respond affirmatively to the question: "Can the health hazard from dermal exposure to toxic chemicals be predicted correctly on the basis of fundamental biological and physicochemical principles?"

Methodology

The approach may be summarized in three components:

1. The establishment of a comprehensive percutaneous absorption database, cross-referenced to physicochemical information on the chemicals studied. The review, collation, and filing for future access: a. human and animal _in vivo_ results, b. _in vitro_ penetration data through excised skin, and c. proposed pharmacokinetic and diffusional descriptions of skin transport and biodisposition.

2. The development of interpretive models for percutaneous absorption; pharmacokinetic and diffusional and structure-activity relationships are being investigated and refined. The models incorporate both established cutaneous biology and recognized physicochemical interactions between the penetrant and skin.

3. The assessment (and initiation) of experimental approaches to transdermal absorption; the identification of optimal methodologies for future determinations of potential cutaneous toxicity.

Progress & Accomplishments

Activity in three areas may be identified:

a. Literature search and database establishment. A comprehensive evaluation of the percutaneous absorption literature has been made and database are being set up on both Apple IIe and IIc microcomputers and on the NIH Prophet resource. Appropriate software has been obtained and accessed. The Prophet system will ultimately allow merging of the percutaneous absorption information with corresponding physicochemical data on the penetrants. The subsequent aim is to utilize developed structure-activity analysis software to define quantitative relationships applicable to dermal transport.

b. Theoretical development of models for the prediction of skin penetration kinetics. Specific descriptions of the chemical input function at the skin surface have been investigated. Zero-order and first-order kinetics have been considered and the approach has been applied specifically to a few very active drug molecules (e.g. nitroglycerin, clonidine), for which well-characterized input functions are available. Good agreement between _in vivo_ data and predictions based upon pharmacokinetic and physicochemical information has been obtained. Although initial progress has targetted transdermal drug delivery as the major application of the analysis, the principles involved are readily extrapolated to occupational exposure situations.

c. Exploration, extension and application of new and existing methodologies for percutaneous absorption assessment. 1. Measurements have been made of the effect of aging on percutaneous absorption in man. _In vivo_ skin penetration studies reveal that there are differences in absorption between elderly subjects and young "controls" and that the differences are greatest for more water-soluble compounds. 2. Multiple-dose experiments have been performed to evaluate the predictive potential of simpler acute exposure measurements. Good correlation between penetration, surface recovery and penetrant pertitioning characteristics has been obtained. 3. Structure-penetration relationships for a series of nicotinic acid esters have been derived in model, _in vivo_ and _in vitro_ percutaneous absorption studies. Extension of the work with a range of phenolic derivatives is in progress.

Significance

Skin disease, initiated in the work place, currently represents a major occupational health problem in the United States. Prediction of the detrimental toxic effects of hazardous chemical exposure is difficult, however, because of the complexity of the percutaneous absorption process and a lack of any consistently identifiable relationship(s) between transport rate and chemical properties. In addition, the very diverse approaches, which have been used to measure skin penetration, further complicate the problem since the extrapolation of results to the human _in vivo_ situation is an exercise requiring often quite unreasonable and, hence, potentially dangerous assumptions. The ultimate goal of the research proposed here is to address this area of occupational health concern. Therefore, our specific aim is acquisition of the ability to predict accurately the toxicokinetics of occupationally-encountered molecules absorbed across human skin _in vivo_.

Publications - Abstract(s) available

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PROTECTIVE CREAMS TO PREVENT POISON OAK/IVY DERMATITIS

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Objectives

This study was designed to test various preparations in an attempt to develop a topical preparation which will prevent poison oak/ivy dermatitis when applied to the skin prior to exposure to the plants.

Methodology

Subjects were initially patch tested to various dilutions of a poison ivy extract to verify their sensitivity and to determine how sensitive they were. Subsequently, patients were patch tested to a specific concentration of the extract (determined by the prior testing) at 16 different sites on the back. At 15 of the sites various potential protectants were rubbed into the area prior to applying the extract.

Those preparations which abolished or signifcantly reduced the dermatitis were then evaluated in a wash-off study, since it was felt that the conditions of the regular patch test were quite stringent and unlike the conditions which would exist during actual use. During this phase of the testing, test patches and controls were performed in triplicate. One set of patches was removed 8-12 hours after applications (similar to what would happen during clinical use of a protectant), a second set was removed after 24 hours, and the final set was removed after 48 hours (as was done in the original testing).

Progress & Accomplishments

Our first accomplishment was the development of the closed patch test procedure which enabled us to rapidly screen the potential efficacy of a wide variety of substances. This system was used to conduct tests of several classes of substances in simple vehicles (ethanol, water petrolatum).

Polyvinylpyrrolidone (PVP) and two PVP-quaternium ammonium derivatives, two commercially available "protectants" (Toxic Guard, Prevent), and a variety of nucleophiles (cysteine, cysteamine, glutathione, and hypotaurine) were tested and found to have little or no efficacy in preventing the dermatitis. Several polyamine sales of linoleic acid dimer (hereafter called dimer salts), however, were found to be beneficial. The most effective dimer salts contained one of several different polyoxyproplyleneamines which go by the trade name "Jeffamine". In general, 20 or 35% concentrations of the dimer salts were necessary for significant protection to be evident. 65% or more of subjects were protected from a dermatitis with the 35% dimer salts in pertrolatum which were made with either Jeffamine D400, D230, or T403. Increasing the ratio of the polyamine to the linoleic acid dimer did not cause a consistent improvement in protection, suggesting that protection is not the result of a chemical reaction between the poison ivy antigen and the amine groups of the protectant.

Several of the most effective preparations (eg. Jeffamine D400 dimer salt, 35% in petrolatum and in ethanol; Jeffamine D230 dimer salt, 35% in petrolatum) were then evaluated in the wash-off trials and in several cased the protection could be increased to 78-90% of subjects if the sites were washed off within 8-12 hours.

Mixing experiments were performed to try to further elucidate the mechanism of action of the protectants. One of the protectants (Jeffamine D230 dimer salt, 35% in ethanol) was mixed with the poison ivy antigen in four different ratios and at three different time intervals (15 minutes, 2 hours, 24 hours) prior to taping the mixtures to the back. As in the other tests, the results were most consistent with the protectant acting as a physical barrier, rather than as a chemical detoxifier.

Two subjects were evaluated to see if the protectants were useful with other allergens, but protection was not noted.

Significance

The results of this study indicate that several polyoxypropyleneamine salts of linoleic acid dimer can prevent poison ivy dermatitis in a significant percentage of subjects, probably by acting as a physical barrier. Insufficient testing has been done to comment on its potential usefulness for other allergens. Further studies on more pleasing formulations, potential toxicity, and long-term use need to be performed. If successful, however, such a product will prove to be a real boon to the many individuals who are allergic to poison oak/ivy.

Publications - Abstract(s) available

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TRANSDERMAL KINETICS AFTER MULTIPLE CUTANEOUS EXPOSURE

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Dermatologic Conditions
5 R03 OH01830-02
07/01/83 - 12/31/85
$19,590 ($37,851 Cum)

Objectives

The long-term goals of the project are:

A. To indentify whether multiple cutaneous exposure can be reliably estimated from single-dose percutaneous absorption experiments.

B. To develop a physicochemical model for skin penetration kinetics that, ultimately, will allow prediction of a penetrant's potential dermal toxicity on the basis of (i) its chemical properties, and (ii) the structure of skin.

Methodology

In vivo multiple dose percutaneous absorption studies have been conducted in rhesus monkeys and in men. The basic protocol involves monitoring the urinary excretion of ^{14}C-labelled penetrant over a period of 14 days. The radiotagged molecule is applied on days 1 and 8; on days 2-7 and 9-14, "cold" chemical is administered to the same skin site at the same surface dose. The effects of multiple dosing are assessed by comparing the ^{14}C-elimination profiles to that observed following a single exposure. In rehesus monkeys, benzoic acid, parathion and salicylic acid have been studied. ^{14}C-excretion following intravenous dosing of chemical has also been measured to provide appropriate correction factors for incomplete renal elimination. In man, attention has been focussed on hydrocortisone, estradiol and testosterone. Single and multiple-dose percutaneous absorption experiments have been conducted with and without occlusion of the site of chemical application. Occlusion is achieved with a polypropylene (Hilltop) chamber. Recovery of the applied ^{14}C-dose(s) is possible and good mass balance has been demonstrated.

The thrust of the model development work centers upon the extension and application of a physicochemical-pharmacokinetic simulation for transdermal delivery and percutaneous absorption. The key rate parameters in the model are predicted from (a) diffusion theory and the penetrant molecular weight, and (b) the octonal/water partition coefficient (K) of the substrate. Validation of the approach has been achieved by fitting *in vivo* data for skin penetration of a broad range of organic compounds. Extension of the model to address (i) specific input kinetics from the topical "delivery system", (ii) the effect of surface loss processes (e.g. volatilization) on absorption, and (iii) the interpretation of *in vitro* penetration darta, has been considered.

Progress & Accomplishments

Progress toward the two basic goals of the project and in a third "exploratory" area has been achieved.

1. The *in vivo* multidose experiments in both rhesus monkeys and man have shown that a single acute exposure and measurement of percutaneous absorption is predictive of penetration following repeated application. No significant alterations in absorption have been observed for any of the compounds

considered when repetitively dosed over a two-week period. Experiments under occlusion have demonstrated significant promotion of absorption for estradiol and testosterone but not hydrocortisone. A clear inverse correlation between surface recovery of penetrant and ultimate absorption has been established; equally, penetration increases linearly with log K for the three steroids. less invasive and more direct determinations of acute and chronic dermal exposure to chemicals are therefore suggested.

2. The physicochemical-pharmacokinetic model of percutaneous absorption developed in this work has been validated through its application to the interpretation of (human) in vivo penetration data for a wide range of organic compounds (including aspirin, benzoic acid, caffeine, chloramphenicol, colchicine, dinitrochlorobenzene, diethyl toluamide, malathion, nicotinic acid esters, nitrobenzene, and salicyclic acid). Subsequently, the model has been extended to include specific input kinetics from the delivery system. Using transdermal drug delivery examples of nitroglycerin and clonidine, successful modelling of the resulting plasma concentration vs. time profiles has been possible. Surface disappearance through volatilization (which is relevant to dermal exposure to organic solvents, for example) has been incorporated into the simulation and the ramifications of the process determined. Further efforts have been directed toward the assessment of in vitro skin penetration data, in particular to address chemical delivery in volatile solvents which may, themselves, alter the kinetics of absorption.

3. Additional activity pursued and progress established during this project includes: (a) Investigation of local chemical delivery into skin in vivo by laser Doppler velocimetry. Pharmacologically-induced changes in skin blood flow have been used to elicit non-invasively details of the kinetics and extent of percutaneous penetration. (b) Calculations of body exposure from skin absorption data have been reported. Simple predictive relationships have been proposed and tested using various exposure situations. (c) Chemical delivery to local subcutaneous structures following topical contact has been reviewed. The extent and kinetics of this event have been considered and have been found to be more significant than previously believed.

Significance

The percutaneous penetration of chemicals accounts for a significant number of toxic observations. The ability to predict and hence minimize exposure and toxic potential is routinely based upon acute exposure and a single-dose transdermal absorption determination. However, the more environmentally and occupationally relevant situation often involves multiple cutaneous exposure for which very little experimental investigation has been performed. The project aims to resolve this insufficiency by: (i) conducting chronic percutaneous absorption studies, and (ii) developing a pharmacokinetic model for interpretation and prediction of transdermal penetration kinetics following topical application.

Publications

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Bucks DAW, Maibach HI, Guy RH: Percutaneous absorption of seroids: Effect of repeated application. J Pharm Sci 74:1337-1339, 1985.

OCCUPATIONAL HEALTH HAZARDS IN SKIN INJURY BY MITOMYCIN C

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Dermatologic Conditions
1 R03 OH01990-01A1
04/01/85-03/31/87
$20,804 ($20,804 Cum)

Objectives

The study of factors controlling the generation of cutaneous intradermal injuries from the antitumor agent MMC. This drug is a health hazard for occupation related injuries in health care workers, and treatment related injuries in cancer patients. Current and projected usage of MMC show a substantial potential risk of cutaneous injury and present treatment methods are unsatisfactory. A murine model will be used, and factors of injury generation will be studied: dose effects, injury generation time, healing time, and drug activation rate. Treatment measures that will reduce, prevent, or eliminate, cutaneous injury from the use of MMC will be studied. Since heat has been reported to potentiate the in vitro antitumor cytotoxicity of MMC, it is hypothesized that antidotal attempts with heat will worsen ulcers, and heat and cooling antidotal effects will be studied.

MMC lesions appear to resemble adriamycin cutaneous lesions. Since adriamycin induced cutaneous lesions have been reported to respond to DMSO treatment, it is hypothesized that DMSO will be effective against MMC induced cutaneous lesions. Furthermore, the reports of DMSO's effectiveness, when applied distally to the ulcer site, will be evaluated in MMC induced ulcers.

Methodology

Skin Toxicity Studies: Dehaired mice were given topical MMC intradermal (ID injections at various doses, and assessed for cutaneous toxicity. Topical antidotal applications of sodium chloride, sodium thiosulfate, N-acetylcysteine, catalase, heparin, hyaluronidase, hydrocortisone, superoxide dismutase, diphenhydramine, fumaric acid, lidocaine, isoproterenol, alpha- tocopherol, cysteine, and DMSO, were made following ID exposure and evaluated for efficacy against MMC. Because of the marked antidotal activity observed with DMSO, time dose-dependent studies were pursued to determine the optimal DMSO application schedule, and the effects of delayed DMSO application. Daily ulcer measurements were integrated to give area under the toxicity x time curve in cm2 days, and appropriate statistics (eg. ANOVA) performed.

Progress & Accomplishments

Skin Toxicity Studies: MMC produced dose-dependent toxicity. Ulcers appeared within 1-5 days following injection, with induration and erythema 20-fold and 10-fold respectively greater in size than the central ulcer. Erythema tended to peak by day 5, and usually resolved over a 2-4 week period. Induration also peaked at day 5, but required up to 7 weeks to completely resolve. MMC ulcers had a central necrotic appearance and resembled adriamycin induced ulcers. MMC did not produce uniformly larger ulcers with larger doses, but larger doses did require longer healing times.

Several potential antidotes significantly increased MMC ulceration. These included: N-acetylcysteine; diphenhydramine; lidocaine; and both enzymatic treatments - catalase and superoxide dismutase. Sodium thiosulfate reduced MMC ulcers, but the level of antidotal activity did not reach statistical significance. Other antidotes were ineffective at reducing MMC ulceration. These include: hyaluronidase; hydrocortisone; fumaric acid; heparin; several sulfur nucleophiles; isoproterenol; and vitamin E. Neither heating nor cooling of the skin reduced MMC ulcers.

The same antidotal pattern was evident with a higher dose of MMC. No antidotal activity was seen for most of the numerous ID pharmacologic agents evaluated. This parallels exactly, the response patterns seen with the lower dose ID MMC experiments. The in vivo results for heating of MMC ulcers are consistent with the in vitro cytotoxicity observations showing synergism between heat and MMC. Prolonged heating markedly increases the peak ulcer area from ID MMC.

In contrast, the nonpolar solvent, DMSO, applied once after ID MMC, provided complete protection against MMC ulceration following the low dose. More frequent topical applications of DMSO were detrimental and some ulceration was produced after low dose exposure. For the high dose MMC, a single topical application of DMSO immediately after MMC, was again the most effective way of reducing MMC-induced skin ulcers. More frequent DMSO applications were also detrimental against the high dose injections. There was no evidence for systemic antidotal DMSO activity. DMSO antidotal activity was still observed 4 hours after ID MMC, but did not produce statistically significant reduction of toxicity. Delayed application of DMSO for more than 4 hours after MMC completely eliminated any antidotal activity.

Significance

Reliably produced MMC ulcers in mice have been demonstrated, and the characteristics of healing profiled. A large number of potential MMC antidotes were found to be inactive. Although sodium thiosulfate showed some antidotal activity, the magnitude of the benefit is smaller than that observed for DMSO. The agents that produced no benefit can not be recommended as possible clinical antidotal treatments. Furthermore, an additional number of agents increased MMC toxicity, and should be specifically avoided in clinical settings where MMC cutaneous toxicity has occurred.

DMSO appears to give substantial protection against MMC-induced skin ulceration in the mouse when applied immediately after exposure and proximal to the ID site. The results of the DMSO treatments show that some antidotal activity exists even when given up to 4 hours after MMC ID exposure. This agent may prove to be efficacious in clinical settings with further study.

Publications - None to date

THE EFFECTS OF PHENOLS ON CUTANEOUS IMMUNE REACTIVITY

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Dermatologic Conditions
1 R03 OH02091-01
04/01/85-03/31/87
$22,387 ($22,387 Cum)

Objectives

Phenols have complicated effects on biological systems. For example, phenols with nucleophilic side chains in the para position (parasubstituted phenols -- PSP) applied topically to the skin induce proliferation of keratinocytes and pigment cells. PSP markedly alter the numerical density of Ia positive epidermal Langerhans cell populations, i.e., increase or decrease their number depending on the dose of PSP applied. Langerhans cells (LC) are immune macrophages in the epidermis. Their surface Ia markers seem essential for their interaction with T-lymphocytes and for initiation of immune reactivity. Alterations in Ia positive cells by PSP probably will alter cutaneous immune reactivity. From other work completed, we suggest one mechanism by which PSP alter LC is by initiating the oxidation of arachidonic acid to prostaglandins and/or leukotrienes. The objective of this proposal is to study the effects of PSP and prostaglandins on cutaneous immune reactivity _in vitro_ and _in vivo_. Commonly available compounds like 4-hydroxy anisole, monobenzyl ether of hydroquinone, and butylated hydroxytoluene will be applied to the skin of mice. Tissue will be stained by a variety of techniques, and alterations in the number and morphology of LC determined. Animals with excessive or depleted LC will be contact sensitized to DNFB to determine whether these agents alter immune reactivity. Skin of animals known to be sensitive to DNFB will be depleted of Ia positive LC and challenged to the antigen in order to determine whether LC have a role in the efferent cutaneous inflammatory response. Epidermis with augmented or depleted numbers of LC will be made into single cell suspensions. The ability of LC in these suspensions to present antigen to syngeneic or allogeneic T-cells, to activate allogeneic T-lymphocytes (MLR) will be measured by _in vitro_ techniques. As a result we will perform a series of studies to determine the effects of prostaglandins, thromboxanes, and leukotrienes on the function of Langerhans cells _in vitro_ and _in vivo_.

Methodology

i) _Screening of Phenols: Identification of PSP which alter the population density of Langerhans cells (cells/mm^2)_. We have tested some of the more common phenolic agents such as 4-hydroxy anisole, monobenzyl ether of hydroquinone, and butylated hydroxytoluene on the population density of Langerhans cells. The compounds in low (.001%) to high (10%) concentrations in 95% ethanol diluent were applied to the ears and back of DBS/2 or C57BL/6 mich. The epidermis from treated and control groups of mice were surgically excised and, following a two-hour incubation in .020 M Na EDTA, the resulting epidermal sheets were stained for Ia positive Langerhans cells by either indirect immunofluorescence, indirect immunoperoxidase or ATPase staining.

2) Sensitization and Elicitation of Contact Sensitivity. Mice treated with the various PSP at various concentrations to increase or decrease the number of Ia positive cells were contact sensitized with dinitrofluorobenzene or oxazolone on the treated sensitization site (back) and challenged on the ears five days later. An ear swelling assay was used to measure the intensity of the in vivo immune response for both afferent and efferent (inflammatory) contact hypersensitivity responses.

3) In Vitro Langerhans Cell Functional Assays. Single cell suspensions of treated and control epidermal sheets were prepared using trypsin and DNAse I digestion techniques. Access to a fluorescence activated cell sorter (FACS) will supplement our previously described (original proposal) Hypaque Ficoll technique to enrich for Ia positive LC. Enriched Langerhans cell suspensions (= 85%) obtained from various treatment groups were observed for in vitro accessory cell function, including both MLR and mitogen stimulation studies using splenic T-cell enriched responder cells. In addition, immunoelectronmicroscopy studies of prepared epidermal cell suspensions were undertaken to confirm ultrastructurally the presence of Ia+ dendritic cells.

4) Determination of Whether the Effects of PSP are Mediated by Prostaglandins, Leukotrienes or Other Products of Arachidonic Acid Metabolism. Briefly, to determine if some of our previous findings observed following topical application of various PSP were being mediated via arachidonic acid or its metabolites both in vivo and in vitro experiments as described above will be performed using various prostaglandins and leukotrienes.

Progress & Accomplishments

Since funding began ten months ago, a large volume of useful and interesting data has been accrued. We have completed our in vivo experiments involving monobenzyl ether of hydroquinone, 4-hydroxy anisole, and butylated hydroxytoluene. MBEH at a 20% concentration when topically applied produced a significant increase in identifiable Ia positive and ATPase positive Langerhans cells with an associated increase in intensity of the contact hypersensitivity response (see enclosed preprint).

Low doses of BHT (.001%) and 4-hydroxy anisole (1.0%) when topically applied to C57BL/6 mice produced a significant increase in Ia+ Langerhans cells for 4-hydroxy anisole treated mice only. Large concentrations (5.0%) BHT and 4-hydroxy anisole (10.0%) did not produce significant changes in the density of Ia+ cells/mm^2. There were no significant changes in contract hypersensitivity reactivity to BHT and 4-hydroxy anisole. In vitro studies of LC enriched treated epidermal cells are planned for the upcoming year which should provide additional data on the functional aspects of these epidermal macrophages following phenolic compound exposure.

To further examine potential biochemical mechanisms involved with our original PSP hypothesis, MBEH was replaced with a biologically relevant molecule, arachidonic acid. Low doses of arachidonic acid (.05%) produced a significant increase in Ia+ and ATPase+ cells along with an enhanced ear swelling in our contact hypersensitivity experiments. In contrast, a large concentration of the arachidonic acid (2%) produced a dramatic reduction in Ia+ and ATPase+ cells which correlated with suppression of contact hypersensitivity to the contact allergen DNFB.

Since this proposal was submitted as of June 1, 1984, an additional newly identified dentritic epidermal cell has been discovered and is currently under investigation by several laboratories including our own. The cell is an Ia negative dendritic cell which expresses Thy-1, Ly-5 and GM1 surface markers. The function of this cell is unknown, although it may serve as a suppressor or natural killer cell of the skin.

We have begun a series of preliminary studies in which C57Bl/6 mice have been topically treated with BHT and 4-hydroxy anisole in an attempt to identify any morphological or density changes in this cell. At low concentrations (.001%) BHT and (1%) 4-hydroxy anisole, the density of identifiable Thy-1+ cells (cells/mm^2) increased by 50% vs. diluent treated animals.

Additional studies are planned using our parasubstituted phenolic compounds in an attempt to identify the function role of this Thy-1+ dendritic epidermal cell in vivo. In addition, we made the observation that our C57BL/6 mice treated with these compounds did not demonstrate changes in our in vivo DNFB contact hypersensitivity experiments, while our DBA/2 mice exposed to MBEH demonstrated dramatic changes in immune reactivity in vivo. Further, it should be noted that normal DBA/2 mice have a paucity of identifiable Thy-1+ cells vs. naive C57BL/6 mice.

Significance

Langerhans cells are believed to plan an important role in initiating contact allergic reactions in the skin such as poison ivy or a variety of industrial related skin diseases. Our study is designed to establish whether common phenolic compounds can enhance or suppress cutaneous immune reactivity by direct or indirect mechanisms which may modulate Langerhans cell function. Completed in vivo studies using topically applied MBEH and 4-hydroxy anisole both showed a significant increase in the density of identifiable Ia+ LC. In addition, MBEH treated mice revealed a more vigorous afferent and efferent suggest (potentially) that PSP exposure may actually exacerbate allergic contact dermatitis.

The recent discovery of an additional dendritic bone marrow-derived epidermal cell increases the skin's complexity with regards to normal cutaneous immune function. This cell may potentially serve as a suppressor cell and may either directly or indirectly affect Ia antigen expression on the Langerhans cells or even keratinocytes. Clearly, in light of our findings with regards to the function of the Langerhans cell following parasubstituted phenolic exposure, additional in vivo and in vitro studies are needed to assess the role of the Thy-1+ dendritic epidermal cell following phenol exposure.

It is our consensus that results of this initial study will provide new and important information on the effect of common phenolic compound exposure on the cutaneous immune response and may have useful applications for preventing or treating allergic contact dermatitis in the home and work place.

Publications - Abstract(s) available

ZINC AND CALCIUM EFFECTS ON GUINEA PIG NICKEL DERMATITIS

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Dermatologic Conditions
1 R03 OH02182-01
09/27/85 - 08/31/86
$21,602 ($21,602 Cum)

Objectives

The overall objective of the proposed research is to provide information about the role of the essential minerals calcium Ca) and zinc (Zn) in the development and prevention of nickel (Ni) allergic contact dermatitis (ACD), using the guinea pig model. Nickel sulfate (NiSO4) is a leading cause of ACD in the human populations of North America. Based upon its position in the first transition group of the periodic table, Ni is predicted to interact directly with Zn and indirectly with Ca (via Ca:Zn direct interaction) in biological systems. Little is known about the normal metabolic interactions of the three essential minerals Ca, Ni, and Zn; even less is known about such interactions in specific disease states. Zinc is a keratogenic element. Nickel:Zn and Ni:Ca interactions and associated skin diseases have been noted during animal feeding trials. Calcium:Zn interactions have been noted in Zn-deficiency skin disease of swine. Oral Ni toxicity in chicks has been prevented by increased dietary Zn. Although current topical, intramuscular and dietary restriction approaches to the treatment and prevention of Ni-induced ACD are not usually practical or effective, trials using dietary supplements of Zn or Ca have not been reported in the literature. By selecting one essential macro-element (Ca) and one essential trace element (Zn) that appear to interact with Ni in vivo, the proposed research will investigate whether supplemental intakes of these two minerals influence the development of ACD due to Ni exposure. The specific research aims are to: 1. to produce ACD in guinea pigs with both NiCl2 and NiSO4, 2. to quantitate immunologic changes associated with nickel ACD in the guinea pig by intradermal skin test and labeled thymidine incorporation in the lymphocyte transformation test (LTT), and 3. to investigate the possibility that supplemental oral Zn and Ca may affect the degree of ACD expression in this animal model. The hypotheses tested are: increased oral Zn supplements will reduce or prevent Ni-induced ACD (via a direct Zn:Ni interaction); increased oral Ca supplements will either not affect (Ca, Zn, Ni independence) or will exacerbate Ni ACD (indirect Ca:Ni interaction, via Zn).

Methodology

Non-pregnant female Hartley strain guinea pigs, average weight 200-250 grams, are purchased from Harlan Sprague Dawley, Inc. in lots of six. They are individually identified by placing colored carpet thread in an ear and provided commercial pelleted feed and tap water ad libitum for 7-10 days. After this conditioning period, they are randomly assigned to the following groups: 1) 250 μg $ZnSO_4$/ml deionized distilled water (DDW), 2) 500 μg $ZnSO_4$/ml DDW, 3) DDW, 4) 500 μg $CaSO_4$/ml DDW, 5) 1,000 μg $CaSO_4$/ml DDW, and 6) tap water. At the end of 4 weeks, they are each sensitized, using the allergen ($NiSO_4$) in Freund's complete adjuvant in a protocol of intradermal and topical exposure. After 21 days they are each tested for delayed hypersensitivity by intradermal injection of 0.1 ml of the allergen (e.g. $NiSO_4$) at concentrations of 0.5%, 0.25%, 0.125%, 0.0625%, and 0.03125% and

0.1 ml of isotonic saline as a control. These tests are read at 48 hours by measuring the diameter (mm) of any observed erythema and/or induration at the injection site. The following day each guinea pig is anesthetized and bled via cardiac puncture. Lymphocytes are separated on a discontinuous Ficoll-Hypaque Gradient (1.085 sp. gr.), washed, placed in microtiter culture plate wells, and cultured in RPMI 1640 medium for 5 days at 37'C with 5% CO_2.

Mucophytohemagglutinin (PHA-M), a nonspecific mitogen, and three or more dilutions of the allergen (e.g. $NiSO_4$) are used in the lymphocyte transformation test. During the last 18 hours of incubation, 0.5 µCi of tritiated thymidine (^{3}HTdR) is added to each well. The cells are harvested onto glass-fiber filters with a semi-automatic harvester and measured for incorporation of ^{3}HTdR in scintillation vials containing a toluene-based cocktail in a scintillation counter. Stimulation indices are computed by dividing the arithmetic mean of quadruplicate determinations of counts per minute for stimulated wells, by the arithmetic mean of quadruplicate determinations for unstimulated control wells.

Progress & Accomplishments

The ACD guinea pig model has been established in our laboratory. The work thus far has focused on $NiSO_4$ as the allergen. PHA-M is used for quality control in each LTT. The optimum conditions for lymphocyte transformation test using PHA-M were found to be: 0.5 µg PHA-M per well, flat-bottom (well) microtiter plates, and 1×10^5 lymphocytes per well in a 5-day culture. Of five $NiSO_4$ concentrations, ranging from $1.08 - 27.00 \times 10^{-6}$ M, the following concentrations gave the highest counts per minute: 1.08, 2.70 and 5.40. The 10.80 and 27.00 concentrations appeared to be toxic and were eliminated. Three of the five sensitized control guinea pigs yielded stimulation indices greater than 2.

For the experimental test of the effects of $ZnSO_4$ and $CaSO_4$ supplemented DDW on ACD expression, 32 guinea pigs have completed all measurements, i.e. approximately 5 animals in each of the 6 treatment groups. Thirty-two other guinea pigs are in various stages of the protocol. Preliminary examination revealed that the stimulation indices for the $NiSO_4$ tested animals receiving $ZnSO_4$-supplemented DDW were lower than the DDW controls; the $CaSO_4$-supplemented DDW were higher than the DDW controls. These preliminary observations must yet be confirmed with a larger sample size and statistical tests of significance.

The skin test results on these 32 guinea pigs do not appear to have any pattern. In fact, the responses for the sensitized animals are often no larger than those of the non-sensitized animals. No attempt has yet been made to correlate the skin test and LTT results.

Significance

These preliminary results help to refine the LTT for use in the ACD guinea pig model. The high proportion of sensitized control animals with stimulation indices greater than 2 indicates that this is a desirable model and test system. The poor $NiSO_4$ stimulation of $NISO_4$ sensitized lymphocytes when the LTT concentrations of $NiSO_4$ were high (10.80 and 27.00 x 10^{-6}M) is consistent with other observations of cell toxicity attributed to nickel.

If subsequent testing confirms that $ZnSO_4$ added to the DDW prevents ACD, this effect should be further tested in other animal species and man. Increasing intake of zinc by low-level ingestion is a practical approach and could greatly reduce the incidence of nickel ACD, which is now the most frequent form of ACD in the United States.

The preliminary observed effect of supplemental $CaSO_4$ in the DDW on $NiSO_4$ stimulation of guinea pig lymphocytes, suggest that a Ca:Ni interaction (via Ca:Zn interaction) may be present. This would provide a basis for future studies of possible interactions and better understanding of the in vivo relationship of these elements.

Publications - None to date

OCCUPATIONAL STRESS AND HEALTH OF WOMEN LPN'S AND LSW'S

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Psychologic Disorders
5 R01 OH01968-02
12/01/84 - 11/30/88
$169,759 ($364,893 Cum)

Objectives

The proposed short-term longitudinal interview study of women who are licensed practical nurses (n = 250) and licensed social workers (n = 250) focuses on the relationship between occupational stress and health-related outcomes in women. Building on the investigators' prior research, the study extends our understanding of occupational based psychosocial stress to include not only job conditions and personality traits but also: (a) occupancy of non-workplace roles; (b) the quality of experience in occupational and in non-workplace roles. Quality of experience refers to the balance between rewarding and distressing attributes of a role and is measure by scales developed previously by the investigators.

Two categories of outcomes are examined: (a) physical health indices, including both self-reports and blood pressure measurements; and (b) mental health indices, including role strain, anger, and anxiety.

Major hypotheses concern the effects on physical health and psychological distress of job conditions, occupancy of non-workplace roles, and quality of roles. In addition, possible moderators of the relationship between stressors and health outcomes are examined, e.g. the interactions of work and family roles, and personality variables such as trait anger and anxiety.

Methodology

A stratified sample (race, parental status, partnership status) of LPN's and LSW's, ages 25 to 55, who live within specified zip codes (an approximately 25-mile radius of Boston) was drawn randomly from the professional registries of the two occupations. In individual interviews a variety of measures -- both standard scales and scales developed for this study -- assess role quality (the balance between rewarding and distressing attributes of a role), psychological distress, and physical health, (including blood pressure). During the four years of the project, data will be gathered at three points in time, one year apart. A 10% random sample of subjects is being reinterviewed to establish the test-retest reliability of certain scales, e.g. job conditions, rewards and concerns.

Casual analytic techniques, including LISREL, will be used to evaluate specific hypotheses concerning the effects on health and psychological distress of job conditions, occupancy of non-workplace roles, and quality of experience in roles. In addition we will examine the interactions of occupational and family role variables as these may moderate the relationship between occupational stress and health outcomes.

Progress & Accomplishments

(a) Sampling: As part of the process of identifying and drawing the sample, demographic studies were conducted of women LPN's and LSW's that resulted in a decision to stratify the sample by race, parental status, and partnership status. In this way, the potential confounding of such variables as race and physical health will be avoided. Moreover, because of the low proportion of black women among LSW's, we have developed a census of all black social workers within our geographical area; we need to identify and include all such women to insure adequate numbers for analyses.

(b) Development of Measures: Major investment was made in developing measures of job conditions, social ties, physical health, rewards and concerns of various roles, and costs and benefits of multiple roles. For example, based on research currently in progress by other investigators on women's physical health, prior measures of physical health were modified to include previously neglected stress-related symptoms of women such as urinary tract problems and menstrual difficulties. Futher analyses have been carried out of the rewards and concerns measures previously developed by the principal investigators in order to refine them.

In exploring procedures for determining blood pressure and accessing health records, the decision was made to collect data on blood pressure from all S's and to omit accessing of health records. Because subjects are not being identified by worksite, and because they vary greatly in their life situation, preliminary studies showed that too much uncertainty and diversity existed with respect to obtaining adequate health records. Other problems arose because of the variety of health professionals consulted, faulty memory for details, etc. Interviewers were certified by Red Cross instructors in obtaining blood pressure data; due attention is being paid to issues of reliability and validity.

(c) Pilot Studies: A variety of pilot interviews have been carried out. First, focus group sessions with groups of LPN's and LSW's have been conducted to insure that we assess major aspects of their jobs and other roles and to pretest our measures of these. In addition, individual pilot interviews have been carried out to test the items and instructions for various measures.

Significance

Results of the first wave will be available in the summer of 1986.

Publications - None to date

NEUROPSYCHOLOGICAL EFFECTS OF CHRONIC SOLVENT EXPOSURE

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Psychologic Disorders
1 K01 OH00028-01A1
09/27/85 - 09/26/88
$32,400 ($32,400 Cum)

Objectives

Discrimination of the specific neuropsychological deficits occuring with chronic low-level exposure to different neurotoxic substances and development of a conceptual framework to explicate the mechanisms of these effects are of primary importance in the field of behavioral neurotoxicology. These issues are particularly germane to individual health and welfare as knowledge about them will allow development of screening techniques and preventive health measures aimed at minimizing the neurological, behavioral, social, and safety hazards which may accompany neurotoxic exposures.

This present study will explore these areas by longitudinally examinin a group of workers experiencing chronic exposure to mixed solvents in the silk screening industry. It is predicted that exposed workers will show significant deficits on neuropsychological testing in the functional areas of visuopatial reasoning and short-term memory and that they will have affective complaints. In addition, solvent exposed workers are predicted to have difficulties on tests of cognitive flexibility which are associated with frontal lobe functioning.

Methodology

This three-year study will test a group of workers exposed to a little-investigated group of mixed solvents to be compared with non-exposed workers. Following medical, neurological, and demographic screening, each worker will undergo a short (60-75 minute) battery of neuropsychological tests, including a standard screening battery and additional tests of cognitive flexibility. Retesting will be completed at 12-month intervals.

Research design is modeled after that used in a study on lead-exposed workers in order to maximize comparability of data collected on solvents to existing data on lead.

The present investigation will utilize traditional neuropsychological methodology to examine the specific cognitive deficits which may accompany chronic exposure to this group of mixed solvents. assess relationship of duration of exposure to degree and nature of neuropsychological deficits, examine changes in test performance within subjects over time, and explore the nature of cognitive flexibility in workers with this exposure. Comparisons will be made between the neuropsychological data on this population and those derived from the parallel completed study of lead workers in order to discriminate specific cognitive and affective characteristics of lead and solvent encephalopathies.

Progress & Accomplishments

To date the subject groups to be evaluated for solvent exposure have been identified and agreement to complete the study has been made with management. Preparations have been made to begin testing in 3/86. The data on a lead comparison group have been collected and analyzed.

Significance

Explication of the specific neuropsychologic impairments occuring in workers chronically exposed to a particular group of mixed solvents and comparisons of solvent and lead induced neuropsychologic dysfunction may provide clues to explicating the mechanisms (psychologic, neuropathologic, metabolic) underlying specific neurotoxic encephalopathies. In addition, tasks assessing cognitive flexibility will help determine whether cognitive rigidity may be contributing to the functional intellectual deficits accompanying exposure to these solvents and to gain further information about brain-behavior relationships by examing effects of this exposure on tasks known to be sensitive to cerebral frontal lobe dysfunction.

Publications - None to date

A BEHAVIORAL EVALUATION OF TOLUENE AND ETHANOL

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Psychologic Disorders
5 R03 OH02071-02
09/28/84 - 08/31/86
$23,360 ($47,043 Cum)

Objectives

The contribution of the study is to examine effects of low concentrations of toluene on human abilities which could result in sub-clinical CNS disorders affecting work, performance, or health. Toluene was chosen since it is a common solvent whose air concentrations in industry usually range from 25 to 100 ppm. Acute neurobehavioral effects of toluene vapor are measured by a microcomputer test battery and expressed in "ethanol equivalent units". Forty-two subjects were exposed for seven hours to 0, 75, or 150 ppm over three days. The behavioral evaluation assessed symptoms, visuomotor skills, visual perception, manual dexterity, and short-term memory at 8:00 am, 12:00 noon, and 4:00 pm. The criterion for choosing behavioral tests required prior validation, ease in scoring and interpretation, comparability to tests used in epidemiological studies, and recognized by the National Institute for Occupational Safety and Health and World Health Organization. The acute effects of ingested ethanol were evaluated in an identical manner. Forty-two different subjects were administered 0, 0.326, and 0.648 g/kg body weight, approximating 0.03 and 0.06 blood alcohol concentration. The rationale for including the ethanol component is: 1) it serves as a positive control for the toluene experiment, and 2) it permits a comparison of behavioral effects between solvents.

Chronic effects of solvent exposure have been equated to premature aging. Similarly, it would be useful to express an acute solvent effect by an index of "ethanol equivalent units" since acute neurobehavioral ethanol toxicity is well described.

Methodology

The study was completed in three experiments. The pilot study established the required number of subjects to detect a 5 to 10 percent difference in performance ($a=.05$; $b=.10$) in the toluene and ethanol experiments. It is also permitted an assessment of menthol, the odor masking agent, and an assessment of statistical methods. Subjects were then randomly assigned to one of three orders of exposure presentation in both experiments (0, 75, 150 ppm toluene or 0, 0.03, 0.06 blood alcohol concentration). The results of the pilot study ($n=20$) suggested 41 subjects provided sufficient statistical power. Paid volunteers with at least one year of college, aged 18-35 years old, with no history of solvent exposure, and who consume less that 30 grams ethanol per day will be chosen. Inter-subject variation in solvent absorbance was monitored in breath at each test point, and accompanied by urinary hippuric spot sample. The data analysis will use adjusted scores computed by a multivariate regression analysis. The adjusted scores will then be used in a repeated measures of analyses study design.

Progress & Accomplishments

The three goals of the pilot study were accomplished within the first six months of the study. First, inter-subject variability over the test period suggests that forty new subjects should be chosen for experiments two and three. The actual number of subjects ranged from 20 to 59 subjects on important tests (a=.05; b=.10). Second, the behavioral effects of 0.195 ppm menthol was not detectable on any test results which permits its use as a masking agent. Third, learning trends or improvement over 10 repeated sessions was particularly apparent in tests with a fixed stimulus. The digit span test, Sternberg Test, and Symbol-digit test had significant learning effects. We have since eliminated memorization of repeated digits by randomly generating those numbers. Finally we have identified and signed up a voluntary pool of qualified subjects.

The toluene and ethanol experiments were completed by December, 1985. Forty-one different subjects were used in each experiment. The data is currently being reduced and analyzed. In addition to the performance test scores, each subject's motivation, fatigue, and ethanol tolerance was rated subjectively and objectively. Self-reported symptoms were collected in response to the question "Over the last four hours, what reactions did you have while being tested on the computer?". Observations on sleep and other narcotic effects were also recorded by test administrators.

Accompanying performance scores and symptoms, toluene and ethanol concentrations in the breath were recorded at each test period. The urinary concentration of hippuric acid, a toluene metabolite, was also recorded as a spot sample for each test point.

The data reduction and analysis should be complete by May, 1986.

Significance

Successful completion of these studies should represent an improvement in research methodology by: 1) using a standardized test battery, 2) by introducing an index of solvent potency in ethanol equivalent units, and 3) determining if an acute solvent effect exists at relevant industrial exposures which will aid in interpretation of epidemiological investigations of chronic solvent effects. Most of the test battery has been recommended for use in future epidemiological studies by NIOSH and WHO, thus the results will be useful in the design and interpretation of future studies.

Publications - None to date

REMOVAL OF CONTAMINATING LIQUIDS FROM SURFACES

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Control Technology Research
5 R01 OH001004-04
09/01/80 - 07/31/85
$45,831 ($218,027 Cum)

Objectives

The primary objective was development of an experimental and theoretical understanding of the mechanics of removal of a contaminating liquid from a solid surface. Criteria were sought through which techniques of removal of contaminating liquids can be better understood and, thereby, improved.

Methodology

Thin liquid films were uniformly deposited on by spin coating onto a variety of solid surfaces, and then removed by the shearing and impaction of fluid jets. Techniques developed for measuring the rate of removal of a film from a surface included optical (light transmission) and weight loss methods. Mathematical models were developed using analytical procedures (lubrication theory) as well as numerical methods for solution of the Navier-Stokes equations. The mathematical models provided guidance in the correlation of data in formats which have predictive capabilities with respect to a number of practical issues in cleaning technology.

Progress & Accomplishments

The study of removal of a thin liquid film from a smooth surface has been completed for final film thicknesses down to approximately ten microns. Publications 2 and 5 provide details. In summary, data have been obtained over a wide range of conditions the characterize the contaminant film and jet used to flush the film, and the mathematical models developed in the course of this program agree quite well with the data, and may be used with some confidence for the prediction of cleaning performance.

The role of surface roughness in affecting cleaning has been examined, both experimentally and theoretically, in our studies of contaminant trapped in a geometrically well-defined cavity. Again, mathematical models developed in our program are in good agreement with the data obtained herein. These studies are described in detail in publications 1 and 3.

Secondary contamination arising from droplets formed by the splashing from a jet impacting on a contaminated surface has been investigated, using both uncontrolled as well as controlled jets. The data for uncontrolled jets (Publication 6) demonstrate a strong dependence of the frequency of drop formation on the jet speed. In particular, certain "velocity windows" appear within which drop formation is suppressed.

The detailed mechanism of drop formation has been elucidated by controlling the amplitude and frequency of disturbances in the jet radius. Critical conditions for onset of splashing have been determined, and correlated, in terms of jet parameters. Publication 7 provides details of these studies.

Publications

Middleman S, Chilukuri R: Circulation, diffusion, and reaction within a liquid trapped in a cavity. Chem Eng Commun 22:127-138, 1983.

Middleman S, Chilukuri R, Aeling D: Removal of a thin liquid film from a flat surface using an axisymmetric impinging jet. J Fluids Eng 106:223-226, 1984.

Tighe S, Middleman S: An experimental study of convection-aided removal of a contaminant from a cavity in a surface. Chem Eng Commun 33:149-157, 1985.

Middleman S: Research on cleaning up in San Diego. Chem Eng Education 170-173, Fall 1983.

Middleman S, Yeckel A: Removal of a viscous film from a rigid plane surface by an impinging liquid jet. Chem Eng Commun, to appear 1986.

Middleman S: Mechanism and control of droplets formed by cleaning jets on wafers. Proc Microcontam Conf, San Jose, Nov. 20, 1985.

Middleman S, Errico M: Critical conditions for splashing from an impinging jet. In preparation 1986.

CONTROL OF CONTAMINANTS FROM OPEN VESSELS

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Control Technology Research
5 R18 OH01296-02
09/01/82 - 12/31/84
$61,704 ($117,057 Cum)

Objectives

The objective of this research is to create Computer Aided Design (CAD) procedures for designers to determine whether the contaminant concentrations in the vicinity of the push pull rim exhausters they have designed satisfy OSHA requirements. The specific goals of the proposed research are:

(1) Extend existing computer codes to solve the equations of turbulent flow and predict velocity and concentration fields in the vicinity of open vessels equipped with push pull rim exhausters.

(2) Conduct experiments to verify numerical predictions.

(3) Create computer software and documentation for microcomputers.

Methodology

Open vessels are used in numerous industrial processes in which it is not practical to enclose the vessel. Vapors emanating from the liquid surface are removed by rim exhausters. If air is blown through the slot near the worker and air is withdrawn through the slot on the far side, the system is called a Push Pull System. Push pull rim exhausters are widely used, but little understood devices to prevent vapors from entering the work place. Several variations of rim exhausters are recommended by ACGIH but design criteria are imprecise and in some cases nonexistent.

Publications - Abstract(s) available

A MODEL FOR PREDICTING GLOVE POLYMER PERMEATION

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Control Technology Research
5 R01 OH01932-03
12/01/83 - 11/30/86
$65,366 ($199,089 Cum)

Objectives

The objective of this work is to utilize physical-chemical parameters for solvent polymer systems in models to predict pertinent permeation parameters; most notably the three-dimensional solubility parameter (3-DSP) of the solvent and polymer and the molecular size and shape of the solvent. Using a multiple regression model, these parameters will be regressed against permeation parameters such as steady-state permeation rate and breakthrough time for known polymer-solvent combinations. Then, from the model permeation parameters can be predicted for unknown combinations.

Methodology

Solubility parameters for polymers are determined using a method developed by Hansen in which polymer samples are submerged in approximately 55 different solvents. Those solvents which affect the polymer most will have solubility parameters similar to the polymer. For molecular size and shape of the permeant molecule, it is realized that some combination of the two concepts will have to be used. All permeation tests have been completed using ASTM D-739 test methods. Both infrared and gas chromatographic detection techniques have been utilized.

Progress & Accomplishments

Progress to date has resulted in the construction of a model for Viton^{R} using the difference between the solubility parameters for Viton and 19 solvents regressed against steady-state permeation rate and breakthrough time. For steady-state permeation rate, the correlation coefficient was 0.69 while for breakthrough time the correlation coefficient was 0.65. The results from this model were used to predict breakthrough times and permeation rates for unknown solvents. In addition, upper and lower 95% confidence limits were calculated for permeation rate and breakthrough time respectively. Several other experiments have been completed which relate to methods for relativizing permeation test data with respect to several variables. Both the thickness and temperature have been varied in order to determine the relationship between those two variables and permeation parameters. The relationships for Vitron have, for the most, part, indicated relationships previously shown in literature. A third experiment explored variables regarding breakthrough time. Breakthrough time is a function of both the sensitivity of the analytical instrument and the permeant polymer system. Consequently, it can be somewhat arbitrary. The error caused by differences in analytical sensitivity has been determined. This error can be quite significant. In addition, we have utilized a new concept, accumulation time, to characterize the time necessary for a certain small amount of mass to permeate through the membrane. This parameter will be more consistent from one analytical device to another and should improve modeling techniques used in this grant.

Significance

The results for Viton which will be published soon, indicate clearly the significance of the work. Minimally, from these results, industrial hygienists can predict if Viton will be suitable for their particular application. Until the molecular size variable is added to the model, inaccuracies will remain so that once the industrial hygienist has predicted that a certain glove is appropriate, that glove should be tested. However, since there are at least ten glove polymers on the market, elimination of eight or nine of these by use of this model will be very useful to the industrial hygienist. Work is now underway to apply similar models to butyl and nitrile rubber.

Publications

Perkins JL: Use of the Three imensional Solubility Parameter to Predict Glove Permeation. Am Ind Hyg J 46:455, 1985.

EXPERIMENTAL PNEUMOCONIOSIS OF SANDBLASTING SUBSTITUTES

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Control Technology Research
5 R01 OH01970-02
04/01/84 - 03/31/87
$80,632 ($172,484 Cum)

Objectives

The use of sandblasting to clean steel structures before applying paint is associated with severe and rapidly progressive silicosis. High morbidity and mortality rates have been noted within a few years of exposure. The U.S. Department of Labor has estimated the number of workers engaged in sandblasting to be 78,000. Recognition of these hazards led to the prohibition of sandblasting in Europe and Australia; in the U.S., sand substitutes are now increasingly being used. They are derived from coal ash residues and slag from smelting furnaces, and are comprised of amorphous silicates of Fe, Al, Ca, Mg, and Ti in various proportions. The free silica content is only 0.03%.

The objective of this project is to develop a rat model to assess the pulmonary fibrogenic potential of sand substitutes. Both normal and silicotic rats are investigated after prolonged exposure to the substitutes. Subsequent to exposure, both groups are followed for an additional twelve months. Appropriate non-exposed control rats are maintained for comparisons. Rats are sequentially sacrificed for histologic and tissue mineral content evaluations as well as biochemical assessment of lung collagen content.

Methodology

The first group of rats is exposed by inhalation to a ball-milled dust of the substitute at a concentration of 10 mg/m3 for 6 hrs/day, 5 days/week for 12 months. Silicosis is induced in a second group by three intratracheal instillations, each consisting of 0.833 mg silica dust suspended in 0.3 ml saline (total dose of 2.5 mg). Nine months after instillation, the period required for development of silicosis, these animals are also exposed by inhalation to the substitute dust (same dust concentration as the first group) for 6 months. Control and exposed rats are sequentially sacrificed and evaluated for: (1) lung mineral burden; substitute dust determined by ICP and silica dust by X-ray diffraction, (2) hydroxyproline content of lung tissue; determined by the method of Switzer and Summer, and (3) histology; evaluation by light microscopy of sagital sections of right and left lung lobes. In addition of hematoxylin & eosin preparations, two special stains are also employed, i.e., Gomoris' silver impregnation method for reticulin fibers and Masson's trichrome method for collagen fibers.

Progress & Accomplishments

A laminar flow whole body (64 rats) inhalation exposure system was designed, constructed and tested. The exposure system was found to produce uniform exposure among the experimental animals. The coefficient of variation of the amount of dust deposited in rats' lungs was about 18% (10 rats). A new, simple and consistent method for intratracheal instillation of silica dust was developed and evaluated. The amount of silica dust retained in the lungs was 80% of the injected amount with a coefficient of variation of 13.5% (6 rats). The original substitute granules and the ball-milled dust were analyzed by the ICP method. The data indicated that the ball-milling procedure used in the experiment did not change the chemical composition of the starting material. Histologic examination of lungs obtained from exposed rats (6 months) revealed a non-fibrogenic tissue reaction to the substitute dust at this time. Small distinct foci of alveolar macrophages containing numerous dust particles were diffusely scattered throughout the lung parenchyma. These cellular collections were frequently found in peribronchiolar, perivascular and subpleural locations. Although extracellular particles were also evident, cell lysis, acute inflammation and collagen deposition were notably absent. The biochemical evaluations corraborated the light microscopic findings, and did not reveal a statistically significant difference in the hydroxyproline contents of the control and exposed rat lungs.

Significance

This study will assess for the first time the safety of these substitutes. It is believed that this rat model will have application in delineating and predicting the potential occupational hazard for abrasive blasters with and without pre-existing silicosis. Occupational exposure standards are based on the assumption that the risk of any particular agent is not influenced by exposure to other environmental factors. This study will provide important information on the risk of lung fibrosis from biologic interactions between different inhaled agents, which could affect the future development of occupational standards. More specifically, this project may demonstrate increased risk of progression of silicosis if silicotic sandblasters use the substitutes.

Publications - None to date

OPTICAL STUDY OF LATERAL VENTILATION OF OPEN VESSELS

Gary S. Settles, Ph.D.	Control Technology Research
Pennsylvania State University	1 R01 OH02090-01
303 M. E. Building	09/28/84 - 08/31/85
University Park, Pennsylvania 16802	$39,649 ($39,649 Cum)

Objectives

1) Development of a Theoretical Model of the three dimensional velocity field into a flanged circular hood, both with and without a crossdraft blowing perpendicular to the hood centerline.

2) Empirical validation of the above model.

3) Development of a theoretical model of capture efficiency based on the validated flowfield model using computer aided design.

4) Validation of the capture efficiency model.

Methodology

A modified potential flow solution was developed for the flow field into the flanged circular hood. An empirical validation was conducted using hot film anemometry for hoods operating with and without crossdraft. BASIC programs have been written for the IBM XT and AT personal computers that enable graphic plots of the flowfield to be displayed in two dimensions. These plots were used to construct models of capture efficiency, which were evaluated using a Sulfur Hexafluoride tracer gas system with portable Gas Chromatograph.

Progress & Accomplishments

All objectives are complete except a field validation of the model.

Significance

The work presents a method for assessing the effects of crossdrafts on hood performance and quantitatively evaluating a hood's performance. The potential for determining the adjustments needed to a given system in order to reduce the employee's exposure from one level to another are discussed.

Publications - Abstract(s) available

FUNDAMENTAL INVESTIGATION OF EXHAUST HOODS

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Pittsburgh, Pennsylvania 15260

Control Technology Research
1 R01 OH02132-01
05/01/85 - 04/30/88
$96,059 ($96,059 Cum)

Objectives

The long-term objective of this grant is the optimization of exhaust hoods used in industrial ventilation, maximizing the capture ability and minimizing the air flow through the hood. Such an approach will reduce worker exposure as well as the cost of operating the exhaust system.

Methodology

The theoretical portion of the study utilizes superimposition and conformal mapping techniques to solve the flow field equations for oblong, circular and triangular hood openings. The experimental verification of the theoretical results is carried out by the measurement of airflow using a hot wire anemometer. The flow measurements are carried out in front of specifically designed hoods to represent a wide variety of hoods. The ultimate test of the hoods will be carried out using a tracer gas.

Progress & Accomplishments

To date we have carried out a significant amount of theoretical work and set up the system for experiments. Experimental characterization of hoods is expected to start presently. In the theoretical work, calculation schemes for multiple oblong orifices were completed. In addition, theoretical characterization of arbitrary shapes and specific solutions for circles and triangles are completed. In the preparation for the experiments and calibration of the experimental unit, we have discovered several discrepancies in the accepted literature.

Significance

In the results obtained to date, we were able to show that with minimal effort it is possible to reduce required flow rate for a hood by about 15 percent while increasing the hood capture efficiency to 100 percent under ideal conditions. It is also shown that under non-ideal conditions (cross drafts) the capture efficiency of the hood can be kept at very near to 100 percent with minimal penalty in flow rate. The complete research results should enable designers to apply the principles developed in increasing the economic and capture efficiencies of hoods.

Publications - None to date

CAPTURE EFFICIENCY OF LOCAL EXHAUST VENTILATION SYSTEMS

Michael R. Flynn
Harvard School of Public Health
665 Huntington Avenue
Boston, Massachusetts 02115

Control Technology Research
1 R03 OH02101-01
04/01/85 - 03/31/87
$18,370 ($18,370 Cum)

Objectives

1. Development of a Theoretical Model of the three dimensional velocity field into a flanged circular hood, both with and without a crossdraft blowing perpendicular to the hood centerline.
2. Empirical validation of the above model.
3. Development of a theoretical model of capture efficiency based on the validated flowfield model using computer aided design.
4. Validation of the capture efficiency model.

Methodology

A modified potential flow solution was developed for the flow field into the flanged circular hood. An empirical validation was conducted using hot film anemometry for hoods operating with and without crossdraft. BASIC programs have been written for the IBM XT and AT personal computers that enable graphic plots of the flowfield to be displayed in two dimensions. These plots were used to construct models of capture efficiency, which were evaluated using a Sulfur Hexafluoride tracer gas system with portable Gas Chromatograph.

Progress & Accomplishments

All objectives are complete except a field validation of the model.

Significance

The work presents a method for assessing the effects of crossdrafts on hood performance and quantitatively evaluating a hood's performance. The potential for determining the adjustments needed to a given system in order the reduce the employee's exposure from one level to another are discussed.

Publications - Abstract(s) available

Flynn MR, Ellenbecker MJ: The Potential Flow Solution for Air Flow into a Flanged Circular Hood. Am Ind Hyg Assoc J 46(6):318-322, 1985.

RESPIRATORY HAZARD CONTROL FOR SWINE CONFINEMENT BUILDINGS

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Yoder Incorporated
Kalona, Iowa 52247

Control Technology Research
1 R43 OH01944-01A1
09/28/84 - 05/31/85
$49,875 ($49,875 Cum)

Objectives

Based on field investigations over the past five years, the University of Iowa estimated that from 50% to 75% of the exposed workers and veterinarians working in hog confinement buildings experience some form of adverse respiratory symptoms. The broad goals of this study were to gather data that would lead toward improving the airborne environment to prevent respiratory hazards to animals and humans. Of particular interest to our company is the testing and development of feed modifications as a more cost-effective control for airborne dust and ammonia than traditional but energy consumptive ventilation. The specific objectives proposed for this preliminary (Phase I) study were:

1) to characterize the temporal variability within four similar confinement rooms,

2) to demonstrate that statistically significant reductions in airborne contaminants can be achieved via initially selected control options, and

3) to define the necessary and efficient sampling strategy to evaluate a broader array of control options during future studies.

Methodology

To achieve these goals we proposed that a high technology sampling system be used to continuously monitor environmental gas and dust and to demonstrate the ability of a modified fat content or another feed additive to reduce these levels. The research farm for Yoder, Inc., Kalona, Iowa, provided four rooms, each with a slightly different but comparable ventilation control system, and the potential to formulate and test ventilation control system, and the potential to formulate and test different feeds. The environmental monitoring system was based on electronic aerosol monitors (GCA Mini-RAM, Model PDM-3 which record 8-hour time weighted averages) and gas chromatography (an automated hourly grab-sample for each room) backed up with colorimetric tubes (grab samples collected three times per week) for carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulfide (H_2S). Two six-week farrowing (birth-to-weaning) cycles were monitored and comparisons made both between rooms and between cycles.

Progress & Accomplishments

Ventilation in these buildings is relatively constant only when air temperatures remain below the ventilation cooling thresholds of 60-70' F; cold outside temperatures cause low ventilation rates which in turn cause a decrease in air quality. The combined effects of the funding data (late fall), delays in start-up (equipment delivery and an early major malfunction), and an early warm spring resulted in ventilation rates both much higher and more variable than would have occured during the colder winter months.

Conditions during both cycles reduced the air contaminant level for aerosols to 0.05 to 0.35 mg/m^3, ammonia concentrations to 2 to 6 ppm, and carbon dioxide levels to 0.07 to 0.10%; no hydrogen sulfide was detected. Differences in ventilation rates appeared to reduce mean aerosol levels between cycles circa 50% (while geometric variability was near 100%) in all rooms, even those in which no nonventilation changes were made. A few peak gas values (particularly during certain defineable periods of cold days) approached their respective threshold limit values, but all mean gas values were well below their respective threshold limit values and were either statistically and/or physically unaffected by the nonventilation controls tested; ammonia was in fact circa 40% higher in all mechanically ventilated rooms during the 2nd cycle, presumably due to higher room temperatures.

Significance

A much better understanding was gained of diurnal variation in air quality and of the factors affecting these variations. Based upon a variety of problems with the sampling methods in this particular setting, a recommended modification to the sampling strategy was to utilize fewer but more direct long-term sampling methods in the future (such as passive detector tubes and manifolded asperating filters). It is our intention to conduct a similar study again this winter when air quality is normally much poorer and the reproducibility of ventilation rates is much better.

Publications - None to date

NOISE CONTROL FOR HONING AND OTHER MACHINES - PHASE II

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Control Technology Research
2 R44 OH01951-02
09/15/85 - 08/31/87
$134,800 ($178,231 Cum)

Objectives

The major long-term objective of this program is to provide source noise control for machine tools such that operators will be protected against permanent hearing loss due to noise exposure. The primary aims of this research are:

Develop a noise control treatment for the honing machine,
identify machines with similar noise generating mechanisms, and
develop specific noise control treatments for the selected machines.

Methodology

In our Phase I study, we determined the method by which tonal sounds are generated by honing machines. We also identified a number of potential noise control treatments including: Lanchester dampers, dynamic absorbers, increased structural damping, and increased structural stiffness.

In Phase II, which began September 15, 1985, these noise control treatments are being developed with careful consultation with machine design engineers, damping consultants, and machinery operations specialists. A number of different concepts will be analyzed and tested both in our laboratory and in the field. Only the most successful will be marketed in Phase III.

In addition to the honing machine, we will develop noise control treatments for a number of other machines with the same type of sound generating mechanisms.

Progress & Accomplishments

During the first 15 days of this project (15-30 September 1985), we presented the results of our Phase I work at Inter-Noise 85.

Significance

There are about 18,000 operators exposed to honing noise; this noise exposure can be reduced as a result of the research in Phase II. If the analysis techniques used in analyzing the honing machines are applied to other machine tools, additional employees will benefit from noise control — about 500,000 employees in total.

Publications - None to date

NEW METHODS FOR QUANTITATIVE RESPIRATOR FIT TESTING

Klaus Willeke, Ph.D.
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Cincinnati, Ohio 45267

Respirator Research
5 R01 OH01301-03
09/29/82 - 08/31/86
$71,790 ($205,901 Cum)

Objectives

Quantitative respirator fit testing is required or recommended before workers may enter work environments in which hazardous air contaminants are present. A fit test is performed by measuring the concentration of a test aerosol leaking into the breathing space between the respirator and the wearer's face. At the time this grant proposal was written, di-2 ethyl-hexyl phthalate (DOP) was used as a test aerosol in the conventional fit testing method utilizing a photometer as the sensing unit. DOP, however, was at that time suspected to be a human carcinogen. New methods were, therefore, proposed to ensure safe and reliable fit testing. At the time of grant initiation, field tests by several investigators showed that the actual protection workers achieved through respirator wear was in many situations quite different from the protection predicted through conventional fit testing in the laboratory with no correlation apparent between field and laboratory testing. The P.I. postulated that the difference is due to particle-size dependent leakage through the faceseal of the respirator. It was further postulated, in the presence of fractional leakage, exposure to different aerosol size distributions would yield different protection factors not recognizable by the conventional fit testing technique or any other technique that measures in a narrow particle size range. Before any new respirator fit testing method can be recommended utilizing a specific instrumental measurement technique, basic questions on particle-size dependent leakage into the respirator cavity need to be resolved.

Methodology

Aerosol leakage into half-mask negative respirators was measured for particle sizes from 0.05 um to 5 um. Since this covers a span of three decades, several aerosol generation and measurement systems were developed. A fine aerosol test agent composed of a nebulized corn oil and the smoke from burning incense was used to measure small particle sizes from 0.05 um to 0.3 um. For the larger particle sizes from 0.1 um to 5 um, corn oil aerosol was used when testing with liquid aerosols, and limestone aerosol was used when testing with solid aerosols. The liquid aerosols were generated by different compressed air nebulizers, and the dry aerosols were generated through use of a fluidized bed. Humans wearing respirators were exposed in a specially designed fit testing chamber. The fine aerosol was measured by a system composed of a charging device, an electrostatic aerosol classifier (EAC) and a condensation nucleus counter (CNC). The larger aerosols were measured by an active scattering aerosol spectrometer (ASAS) and an aerodynamic particle sizer (APS). Much development work had to be performed to make the instruments compatible with each other and with aerosols generated. All experimentation was approved by the University's Committee on Human Research.

Progress & Accomplishments

Testing with humans turned out to be considerably more complex and difficult than with lifeless models. The aerosols that have leaked into a given respirator cavity are modified by deposition in the wearer's respiratory system so that the exhaled aerosol is different from the inhaled one, both being measured by the same fit testing instruments which probe the respirator cavity during inhalation and exhalation. Furthermore, the particles grow in the humid environment of the respiratory system and are exhaled at a larger size, decreased in number, and at higher temperature and humidity levels. Nevertheless, reliable and repeatable data were eventually obtained.

Our data show that there is essentially no particle size dependent leakage in the size range 0.2 um to 1 um. Below 0.1 um, diffusion to the leak surfaces removes some particles and a moderate decline in relative particle concentration inside the respirator cavity is noted. Above 1 um, the decline in relative particle concentration is more pronounced.

When holes of known size were made into well-fitting masks, the percent aerosol measured inside the mask for each particle size range decreased as the hole size became smaller. The shape of the leak hole affects the particle size distribution inside the mask. A long slit leaks less aerosols than a circular hole having the same cross-sectional area. Both types of leaks occur in actual respirator wear. For a given probe location leak holes in different locations resulted in measured concentrations that differed by a factor as high as four. For a given leak hole two different probe locations resulted in measured concentrations that also differed by a factor of four. The hole location, hole shape and the probe location did not affect equally the measured percent aerosol inside the mask for all particle sizes.

Significance

Based on our findings, conventional quantitative fit testing has become questionable in its ability to reliably predict the worker's protection to hazardous air contaminants.

Publications - Abstract(s) available

A FUNDAMENTAL STUDY OF RESPIRATOR AIR FILTRATION

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University of Minnesota
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Minneapolis, Minnesota 55455

Respirator Research
5 R01 OH01485-03
09/23/83 - 08/30/86
$98,842 ($269,788 Cum)

Objectives

The principal aim of this research is to study the characteristics of particulate air filters of the type used in respiratory protection devices. Commercially available respirator air filters will be studied to determine their efficiency as a function of particle size in the size range of interest in respiratory protection, namely 0.01 to 10 um. In addition, the resistance of the filter media and their dust holding capacities will also be measured, and the role of electrical charge in the conventional mechanical and electrostatic filters will also be determined. A further aim of this research is to make a comparative study of several filter testing methods based on modern aerosol instrumentation and to develop one or more of these methods to the point where they can be routinely used for filter evaluation.

Methodology

Two approaches have been used to measure the filter efficiency as a function of particle size. In one approach, a monodisperse aerosol is generated by means of a vibrating-orifice aerosol generator or an electrostatic classifier aerosol generator. The aerosol concentration upstream and downstream of the filter is then measured with an optical particle counter or condensation nucleus counter. In a second approach, a polydisperse aerosol is generated by an atomizer and the concentration and size distribution of the aerosol upstream and downstream of the filter measured with a single particle optical counter. From this, the efficiency vs. particle size curve is determined. The flow rate through the filter is also varied to study the effect of flow rate on filter efficiency.

The filter loading study is made by generating a concentrated aerosol by atomization and loading the filter with this concentrated aerosol. Upon reaching a certain loading level, the filter with the loaded dust particles is analyzed to determine their efficiency as a function of particle size. In addition, the filter pressure drop is also measured. From this, the effect of dust loading on filter pressure drop and efficiency is determined.

The theoretical study consists of solving the Navier-Stokes equations on the computer to determine the filter pressure drop as a function several filters parameters. The filter efficiency and the influence of electrical charge on filter efficiency will also be determined numerically. The result will then be compared with the experimental data generated in this program.

Progress & Accomplishments

An experimental system for respiratory filter performance evaluation has been successfully developed during the first year and the system has been applied in the second year to the study of the efficiency and pressure drop characteristics of several commercially available respirator filter. The data are being analyzed for comparison with filtration theory. In addition, filter pressure drop calculation was initiated during the second year for the case of rectangular fibers found in some commercial respirators using permanently charged filter fibers.

During the third year, the focus is on completing the measurement of filter efficiency and pressure drop, and the development of the filter pressure drop and efficiency models. Comparison of the theoretical and experimental results will also be undertaken during the third year. The result of the research will also be documented for publication in journals.

Significance

The end result of this research will be an improved understanding of the characteristics of respirator air filters in general and the availability of improved filter testing methods with potential industrial and practical applications. The research should benefit further respiratory protection device development and encourage the correct use of these devices for health protection in the work place.

Publications

BYH Liu: Performance of High Efficiency Respirator Air Filters. Proc International Symposium-Workshop on Particulate and Multi-Phase Processes and 16th Annual Meeting of the Fine Particle Society. p 45-1, Miami Beach, April 22-26, 1985.

RESPIRATOR PERFORMANCE MODEL FOR PARTICULATES

William C. Hinds, Sc.D.
School of Public Health
University of California, Los Angeles
405 Hilgard Avenue
Los Angeles, California 90024

Respirator Research
5 R01 OH01595-03
09/23/83 - 08/31/86
$62,011 ($164,867 Cum)

Objectives

The overall objective of this grant is to provide a better understanding of the effect of particle size on the performance of air-purifying respirators for protection against particulate exposures. This is accomplished through experimental measurement of filter and leak performance as a function of particle size and flow rate and the use of these data in a computer model to predict overall performance for a respirator with a given size and type of leak as a function of airborne particle size distribution and work rate of the wearer.

Methodology

The first phase involves measurement, in the laboratory, the performance of respirator filters and facial seal leaks as a function of particle size using a mineral oil test aerosol and a high resolution optical particle counter. Filter measurements will be made at 7 steady flow rates from 2 to 150 L/min and 14 particle sizes from 0.1 to 11.0 um. Leakage measurements are made at these conditions for three types of leaks and four leakage rates. The second phase uses these data to develop a computer model that gives total penetration (filter penetration plus leakage) as a function of particle size for any inhalation flow rate, leak type and size. The model will be integrated over the breathing cycle associated with five work rates and over log normal size distributions commonly found in industry. The result gives theoretical protection factors for any particle size distribution and for specific jobs where data on work rate, particle size distribution, and leakage rates are available. The model will be applied in conjunction with existing respiratory deposition models to give protection factors based on total and regional deposition as a function of work rate and particle size distribution.

Progress & Accomplishments

Experimental measurements have been completed for half masks with tube type leaks and are underway for leaks that result when wires of different diameter are inserted in the facial seal. The model is developed to the point where 1) performance as a function of particle size for a given filter and leak geometry can be determined for different work rates and 2) overall performance (mass or count penetration and protection factor) of a leaking respirator can be determined for a given work rate and aerosol size distribution.

Significance

The ability of airborne particles to penetrate the filters and facial seal leaks of air-purifying respirators depends strongly on particle size as does the respiratory hazard associated with these particles. Thus, the actual protection factor obtained for airborne particles will depend on the particle sizes present in the work place environment. The respiratory hazard depends on the efficiency of deposition at various sites in the lung and is affected by both the reduction in concentration and the change in size distribution of inhaled aerosol caused by the respirator. With a few exceptions current respiratory protection factors do not distinguish between exposures to aerosols of different particle size. This study characterizes the effect of particle size distribution on the performance of representative single-use and half mask respirators. No general data exist to describe the performance of respirators as a function of particle size or particle size distribution.

Publications

Hinds WC, Kraske G: Performance of PMS Model LAS-X Optical Particle Counter (extended abstract) in Aerosols: Science, Technology and Industrial Application of Airborne Particles. Liu, Pui, Fissan, Eds, p 31-34, Elsevier, New York. Presented at First International Aerosol Conference Sept. 17-21, 1984, Minneapolis, MN.

Hinds WC, Kraske G: Performance of PMS Model LAS-X Optical Particle Counter in J of Aerosol Science, 17(1) 1986.

IN-MASK MONITORS AND RESPIRATOR BREAKTHROUGH

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230 Albany Street
Cambridge, Massachusetts 02139

Respirator Research
5 R01 OH01603-03
09/23/83 - 08/31/86
$131,948 ($273,591 Cum)

Objectives

The objective of this program is to demonstrate that small, chemically sensitive colorimetric films being developed by Moleculon can be combined with miniature electro-optic sensing technology to provide a compact, convenient and reliable method for monitoring in-mask levels of hazardous gases and vapors in air purifying respirators. Such monitoring can both alert the user to early signs of respirator failure, and provide increased safety for the use of cartridge filters close to, but not beyond, their end of life.

Methodology

Initial research has been devoted to better quantifying the response of formaldehyde-sensitive films, with much emphasis on the thin-film chemistry involved, and the sensitivity and stability obtained. Further work has examined the applicability of the same concept to another hazardous gas, hydrogen sulfide. Currently under investigation is the actual use of the formaldehyde sensing system to detect and monitor end of cartridge life for formaldehyde removing cartridges, with emphasis on the experimental test apparatus involved and accurate supporting measurements of low formaldehyde concentrations.

The chemistry underlying the formaldehyde sensing system is a highly modified, nonaqueous Shiff reaction scheme free from hazardous components. The hydrogen sulfide sensor relies on the reaction of certain bismuth salts with hydrogen sulfide in organic solution, bismuth being unusually nontoxic among the metal ions colorimetrically sensitive to sulfide vapor. Both the sulfide and formaldehyde systems are continuously monitored by a tiny dual-beam spectrometer and its associated electronics.

Progress & Accomplishments

Both the formaldehyde and sulfide systems appear to have the sensitivity required for adequate in-mask and cartridge end of life use. The stability and sensitivity of the formaldehyde sensing film have shown fluctuations requiring extensive chemical and coating research. Tests now under way with real respirators are expected to demonstrate the merit and high utility of this approach.

Significance

There are many industrial settings where the use of respirators is the only immediate option for respiratory protection, yet too little is known about the service life of air purifying cartridges toward particular challenges. This leads to uncertainty in the level of protection provided by current masks, requires the uneconomical disposal of cartridges which may retain more than adequate protective capacity, and limits the certification of protective masks for broader use. The kind of inexpensive, in-mask monitoring under investigation here is intended to address all of these important concerns, and will in time greatly increase our understanding of the factors determining cartridge reliability.

Publications - None to date

COMFORT AND ACCEPTABILITY OF RESPIRATORY PROTECTION DEVICES

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John B. Pierce Foundation
290 Congress Avenue
New Haven, Connecticut 06519

Respirator Research
5 R01 OH01608-03
09/23/83 - 08/31/86
$57,754 ($152,190 Cum)

Objectives

The long-term objective is the development of criteria for the design and construction of breathing devices, such as masks and hoods that protect the wearer against occupational and environmental pollutant hazards, to minimize the unpleasantness and discomfort of such masks to make them acceptable to the wearer. The specific aim of the immediate research is to determine the human response to temperature and humidity inside face masks during rest and moderate work in order to quantify the limits of acceptable mask conditions for the wearer.

Methodology

The responses (subjective and physiological) of six subjects wearing ventilated (24 liters/min) face masks during rest and work of 15 min duration in still air were observed over a wide thermal range of ambient and mask conditions. For rest (1 met), the ambient conditions were 25, 30 and 35°C at a 10°C dew point with mask conditions of 27, 30, 33, and 36°C at medium and high water vapor levels (55 and 90% RH). For work (3 met on cycle ergometer), the ambient was 16 and 21°C and mask conditions were 21, 27 and 33°C with water vapor levels of 61 and 86% RH. The subjects wore trousers and a long-sleeved shirt (0.6 clo). Skin temperatures, heart rate and skin wettedness levels were measured continuously. The subjects' judgements of acceptability, thermal sensation, degree of discomfort, sense of skin moisture, and the difficulty of breathing were gathered 14 and 1 min before removing the mask and 5 minutes after.

Progress & Accomplishments

Temperature and comfort sensations for the whole body were affected substantially by the temperature and humidity conditions of air in the mask. A cool mask made the warm body feel cooler, whereas a warm mask made the cool body feel warmer. The thermal sensation experienced under the mask increased linearly with the measured upper lip skin temperature. This skin temperature was independent of whole body ambient conditions but directly related to the air temperature in the mask. The subjects' judgement of the surrounding environment being thermally acceptable for the whole body was strongly influenced by ambient conditions. Hot environments were less acceptable than cooler ones; however increasing the air temperature and/or humidity in the mask decreased whole body acceptability further. In the mask itself, acceptability decreased with increasing mask air temperature and himidity. Mask temperatures cooler than 33 and 27°C for 1 and mets respectively were 100% acceptable. Mask temperatures greater than these were less acceptable. The loss of acceptability was greater for humid mask conditions. Mask acceptability decreased sharply when thermal sensations under the mask were warmer than neutral.

The subjects perceived breathing to be more difficult with increasing mask temperature and humidity. This perception at rest conditions with a humid mask was significantly higher (more difficult) than for the drier mask conditions at temperatures above 30°C. Breathing difficulty was found to be linearly related to the energy content of the inspired air (as described by the thermodynamic property of enthalpy) above enthalpy thresholds of 88 and 70 kJ/kg for the 1 and 3 met activity levels.

Significance

The significance of this research is that if protective breathing devices can be made that minimize the discomfort or unpleasantness to the wearer, they will be worn more often or continuously and thereby improve the protection of exposed individuals. This research seeks to define the design criteria for such comfortable breathing protection.

Publications - None to date

RESPIRATORS: PHYSICAL AND PHYSIOLOGICAL CONSIDERATIONS

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Texas College of Osteopathic Medicine
Camp Bowie at Montgomery
Fort Worth, Texas 76107

Respirator Research
5 R01 OH01617-02
11/01/83 - 10/31/85
$94,583 ($182,183 Cum)

Objectives

A. To provide descriptive - psychophysiological date of the physical stress and physiological strain imposed while wearing a pressure-demand, air-supplied, full-facepiece respirator during maximal and submaximal work to exhaustion.

B. This descriptive data will be used to provide criterion guidelines for selection of worker capability for wearing a respirator.

C. The physiologically descriptive data will be used to verify a computer simulation program on the work of breathing and to propose design modifications of currently produced industrial respirators.

Methodology

Standard maximal ramp and submaximal treadmill protocols were used to provide physical stress to 38 subjects both with and without pressure-demand respirator wear. Each subject required six days of testing. One day included a complete pulmonary function assessment while the other five days were for treadmill testing during which breath-by-breath assessment of cardiopulmonary variables were made. In addition psychophysical scales of perceived exertion and breathing were used during the treadmill tests. Finally the subjects were required to provide a psychological profile of anxiety, which was used "a priori" to predict the presence or absence of psychological distress.

Progress & Accomplishments

A complete set of data was collected on 38 subjects resulting in a data base containing 560 lines of data for each individual subject. As these subjects varied in age, fitness levels and anthropometric parameters our major aim of providing descriptive psychophysiological data relevant to the working population has been achieved. Subsequent multiple regression analysis will enable us to provide guidelines for selection of worker capability for wearing a respirator. In addition design modification have been suggested and computer simulation of the work of breathing has been accomplished.

Significance

This data base will form the basis of our recommendations for pre-screening workers for respirator wear. In addition, it is apparent from the data that significant changes in current respirator performance characteristics will be required to meet the needs of 95% of the working population. Finally the successful use of computer simulation modelling provides the possibility that certification of specific respirators can be accomplished by simulation alone.

Publications - Abstract(s) available

Raven PB: Clinical pulmonary function and the physiological effects of industrial respirators. J Int Soc Respir Protection 1:1-18, 1983.

Jackson AW, Raven PB: Statistical and research design considerations concerning industrial respirator research. J Int Soc Respir Protection 1:31-40, 1983.

Raven PB: Respirator research, where it needs to go: The man-respirator interface. J Int Soc Respir Protection 1:49-66, 1983.

Stojack WB, Raven PB: Development of a quantitative fit testing program for the Clark County Fire Development. J Int Soc Respir Prot, 2:288-295, 1984.

Stojack WB, Raven PB: Prediction of distress for individuals wearing industrial respirators. Amer Ind Hyg Assoc J 13:263-272, 1985.

OPTIMAL DESIGN OF RESPIRATOR CARTRIDGES

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Respirator Research
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Objectives

Respirator cartridge performance is examined through the fundamental theories of mass transport, as used by chemical engineers and physical chemists, to determine optimal design parameters for respirator cartridges.

Methodology

An experimental system consisting of a vapor generator, dilution air stream, charcoal beds, and vapor detector allows the performance of respirator cartridges to be monitored continuously. Mathematical modeling of the observed performance of respirator cartridges as a function of time permits the geometry of the respirator cartridges to be systematically modified to give optimum performance.

Progress & Accomplishments

We developed, using a microcomputer, a rapid method of determining the basic factors, (i.e., the adsorption coefficient and mass transfer efficiency) which control the performance of a respirator cartridge. This computer program was then used in analyzing the performance of respirator cartridges exposed for extended periods of time to high concentrations of contaminant, in a rapidly flowing stream of air, at humidities as high as 100%.

In the process of analyzing these data, we developed the first satisfactory theory for the performance of a respirator cartridge at extremely high relative humidities. This equation is as follows: $W = W_0 \exp(kE^2)$, where W=gm uptake/gm adsorbent, p=density of liquid solvent, k=a structural constant for the adsorbent, W_0=pore volume of adsorbent, and and E=potential energy for adsorption. The potential energy for adsorption form a humid atmosphere is $E/RT = (1+k_1) \ln (P_s/P) + k_2 \ln (RH/100)$, where P is the vapor pressure of the solvent, P_s is the vapor pressure of the liquid solvent, T is to absolute temperature, R the ideal gas constant, and RH is the relative humidity, and k_1 and k_2 are constants describing the effect of the ambient humidity on the adsorption of the vapors of the solvent. By the above equation, the adsorption coefficient can now be determined at any temperature, vapor concentration, and relative humidity. A very important result of the above theory is that when the RH = 100%, the term containing k_2 vanishes; the resultant equation under these conditions gives a simple correlation between the adsorption coefficient from dry air and from air totally saturated with water vapor. This equation fits experimental data for the adsorption of trichloroethylene at relative humidities ranging from 5 to 85% and concentrations ranging from 300 to 1300 ppm, to within 2% error.

An important prediction from the above equation is that even in an atmosphere saturated with water vapor, a respirator cartridge may have a useful residual efficiency. This was proved experimentally using vapors of xylene, methylethyl ketone, and ethyl acetate. For all three vapors, protection for 8 hours could be shown using a standard size respirator cartridge in a air flow of 20 lpm of air completely saturated with water vapor. The key factor learned from this work is that instead of writing off the use of respirator cartridges at high relative humidities, we should determine the circumstances under which they will be effective. Protection against benzene under the same conditions was studied detail. With the standard respirator cartridge, because of the very poor mass transfer of benzene vapors (number of theoretical plates remaining below ten), protection for 8 hours could not be demonstrated. However useful protection for 4 hours could readily be obtained. Next is was found that a change in the geometry of the respirator cartridge permits a respirator cartridge containing 40 grams of adsorbent to protect against benzene in air at 100% RH for more than 8 hours. This demonstrates the importance of understanding the kinetic factors influencing mass transfer.

Significance

By modeling and designing respirator cartridges to operate under adverse conditions, we increase our confidence in their safe use in more normal circumstances. Particularly important is the equation giving the adverse effects of a high relative humidity. As the experiments with benzene showed, ignoring relative humidity may cause unacceptably high exposure to occur without any warning.

Publications

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A MODEL AND TESTS FOR PREDICTION OF RESPIRATOR ADSORBENT BEHAVIOR

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Respirator Research
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Objectives

The Wheeler-Dubinin adsorption model has been proposed for use in the workplace to predict breakthrough times of activated carbon-containing respirator cartridges. The Wheeler-Dubinin Model has been shown to be an accurate predictor of adsorptive behavior (within + 15%) for small carbon masses (<10 g) with a single adsorbable vapor, low volumetric flow rates (<0.05 L/s) and high inlet concentrations (>10,000 ppm). The purpose of these investigations is to extend the Wheeler-Dubinin Model to realistic conditions of use, i.e. multiple adsorbable vapors (binary vapor mixtures with water as the second vapor), large adsorbent masses (30-40 g), high flow rates (1-0.3 L/s), etc., so that accurate predictions of respirator adsorbent cartridge behavior can be made. Three phases have been planned for these investigations; Phase I involved the development of the large bed system, verification of the model and preliminary investigations with binary vapor mixtures; Phase II included investigations of the stability and reliability of the large bed system, and evaluation of commercial cartride packings reproducibility and the degree of carbon activation. Additionally, preliminary scale-up investigations were performed using small bed parameters for prediction of breakthrough with large adsorbent masses; Phase III investigations include the extension of the binary vapor investigations with the inclusion of additional vapors (other than carbon tetrarchloride) and the effects of reference vapor polarity on predictions. Water vapor was chosen as the second vapor because it is normally encountered at high concentrations (>1000 ppm) in the environment and it has been shown to adversely effect time-to-breakthrough predictions.

Methodology

Carbon adsorbents are first characterized using a reference vapor such as carbon tetrachloride. The characterization process involves the generation of a characteristic curve for the reference vapor and the adsorbent. This can be accomplished using the gas chromatographic technique of Dollimore or the dynamic technique. Once the reference adsorbent has been characterized, it can be used in the application of the Wheeler Kinetic breakthrough model. The kinetic data are developed by passing vapor laden-air through an adsorbent bed. The effluent is monitored until a chosen exit-to-inlet ratio (<0.04) is achieved. These data are then plotted as the natural log of the exit-to-inlet ratio versus the time. From this curve, the rate constant and the kinetic capacity are determined. These data are then used to predict the behavior of other vapors. The effect of water vapor will be determined in two modes, one in which water vapor is pre-equilibrated with the adsorbent and a second which utilizes dry caron. These adsorbents are then challenged with vapor-laden (a binary mixture of the test adsorbate and water vapor at the test concentration) air. Again, the effluent is monitored until a chosen exit-to-inlet ratio (<0.04) is achieved. These data are then plotted as the natural log of the exit-to-inlet ratio versus the time. From this line the rate constant and the kinetic capacity are determined. The data can then be used to predict the behavior of other vapors in the presence of water vapor.

Progress & Accomplishments

Major progress has been made in completion of the first two phases of the proposed work. Initial investigations of the third phase are in progress.

In Phase I, the high flow/large adsorbent mass gas/vapor system for challenging adsorbent was constructed and tested. Initial data were developed on the generation of mixtures of vapors. Preliminary data on the effects of water vapor on adsorption of the standard reference vapor, carbon tetrachloride, have been developed. These data indicate that water vapor at low concentrations (RH's below 50%, 23°C) can adversely effect the predictions of the model.

In Phase II of the investigation, the stability and reproducibility of the large bed system were established. An additional investigation was completed which was focused on the reporducibility of respirator adsorbent packing-volumes and a comparison of the extent of activation of the adsorbents. This demonstrated that commercial activated carbon-containing respirator cartridges from three of the major suppliers are associated with significant differences in the degree of adsorbent activation and in the amount (as determined by the mass of adsorbent) of carbon in the cartridge.

Significance

Currently, there is no reliable method for predicting the adsorptive behavior of respirator adsorbent cartridges in use. Although a number of approaches to this problem have been attempted, none have been demonstrated to be widely successful. The Wheeler-Dubinin model has been demonstrated to be an accurate predictor of adsorbent bed behavior. The continued development of this model will permit its general application in the field, thus filling this void.

Publications - None to date

ASSESSMENT OF RESPIRATORS AMONG AGRICULTURAL WORKERS

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Respirator Research
5 R01 OH01650-03
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Summary

The agricultural sector is both a large (nearly 12 million) and understudied occupational environment. Exposure to complex mixtures of organic dusts and chemicals are responsible for a number of respiratory diseases among agricultural workers - including silo filler's disease, farmer's lung, occupational asthma, and most commonly bronchitis and obstructive airways disease. High rates of respiratory symptoms and airways obstruction have been documented among animal confinement workers and grain handlers. These exposures are often difficult to control, for both technical and economic reasons. Therefore, the use of a respirator in these working environments has become a common request from those adversely affected. Yet, there has been no systematic study of respirator efficacy with exposure to these organic dust and gas aerosols.

Three generic respiratory types will be tested - the single use half mask, the reusable half mask, and the powered air-purifying respirator. Four exposure groups will be tested - 50 swine confinement housing workers, 50 poultry confinement housing or processing workers, 50 grain handlers, and later 12 active farmers with farmer's lung using only the most efficient respirator. Respirator protection factors associated changes in lung function and respiratory symptoms, respirator acceptance, the association between respirator efficacy and acceptance and physical fitness, and the possible effect of smoking will all be evaluated. Ambient and in mask sampling for total dust, endotoxin, and ammonia will provide environmental indicators. Change in respiratory function over an exposure period, change in respiratory and eye symptoms and the worker's response to a questionnaire on various aspects of respiratory acceptance will be the medical indicators. Results of this study should provide the basis for development of a "model" respiratory program for these and other organic dust exposures.

Publications - None to date

RESPIRATOR TOLERANCE

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Respirator Research
5 R01 OH02005-02
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$80,761 ($151,861 Cum)

Objectives

1. To identify the determinants of tolerance to respirator use.
2. To characterize the adaptation to respirator use, including the changes in respiratory pattern and volumes.
3. To develop a measure of sensitivity to respiratory loads using a psychophysiologic approach and to use this method for assessing the effects the effects of respirators.
4. To estimate inaccuracies introduced by the use of mouthpieces in laboratory studies of respiratory function.
5. To compare laboratory measures of respirator effects to actual worksite measures.
6. To assess population variability in pattern of adaptation and respirator-type load sensitivity.

Methodology

The study entails three major components: 1. exercise laboratory studies to evaluate physiologic responses. 2. field studies of respirator use, employing ambulatory monitoring methods. 3. limited study of pattern of respiratory adaptation and of load sensitivity in a relatively large sample. An application of Steven's Law assesses an individual's load sensitivity by relating estimated load to the actual resistance and to peak mouth pressure. Respiratory pattern is determined both by measurements made with a mouthpiece-pneumotachograph method and by use of a respiratory inductive plethysmograph (not requiring a mouthpiece).

In the exercise studies, subjects are studied at rest and at several levels of exercise. Measurements include respiratory flow rates and volumes as well as measures of pattern of breathing. Mean inspiratory flow rate, respiratory duty cycle, and several other parameters describe respiratory drive.

The field studies include the use of an ambulatory FM recording system. The system allows measurement of two channels of respiratory plethysmographic data and two for ekg. Playback is accomplished at a 20x rate. Field studies assess respiratory pattern with and without a respirator in use.

Progress & Accomplishments

The exercise laboratory studies are well underway. A series of protocols has been accomplished varying the nature of exercise and of respirator loads.

Inspiratory loading in the presence of an added dead space load leads to significant adaptation. The mean inspiratory flow rates decrease with concomitant prolongation of the inspiratory fraction of the respiratory cycle. Thus, there is relative maintenance of minute ventilation, particularly at rest. The oxygen consumption did not appear to be significantly affected by such loading, even at moderate to high exercise levels.

Thus, it appears that repirator users may adapt by the use of respiratory pattern which minimizes peak respiratory work.

Study of respiratory load sensitivity as loading due to the respirator occurred. In addition, within subject variability in the measure of sensitivity increased at higher exercise levels. The extent of inter-individual variability was greater than the variance accounted for by the use of a respirator.

The presence of a mouthpiece produced a small but detectable effect on tidal volume and respiratory rate. However, this was present only at rest, disappearing during exercise. Thus, the measurement techniques which use mouthpieces is likely to be quite small in studies involving exercise.

The ambulatory studies have begun. Measurements of respiration and cardiac frequency may be made in non-laboratory settings. We have been able to retrieve data through an analog-to-digital converter from the tape playback unit. However, the data include considerable "noise" on the respiratory channels when the subject performs work involving frequent bending. In the absence of such actions, which probably are compressing the abdominal motion transducer, respiratory pattern may be discerned.

Significance

This work is potentially significant for three reasons: First, it demonstrates that there is a discernible pattern of adaptation with prominent effects on respiratory pattern. This suggests that workers who report discomfort from respirator use may require evaluation of their respiratory control in addition to measurements of "lung mechanics" as currently recommended. Second, the work may have methodologic significance: Load scaling has been shown to be an effective method for characterizing an individual's sensitivity independent of other pulmonary tests (although its significance is still uncertain). Also, the ability to noninvasively measure ventilation during work (within certain constraints) has been demonstrated; this method may be useful in studies of responses to worksite stressors other than respirators; for example, change in respiratory pattern may be a sensitive indicator of chemical irritation. Third, the inaccuracy in previous research of respirator physiologic effects introduced by the measurement techniques is likely to be slight.

Publications - None to date

HUMAN METABOLISM OF HALOTHANE MECHANISMS OF TOXICITY

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Other Occupational Concerns
5 R01 OH00978-06
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$211,935 ($920,710 Cum)

Objectives

We are trying to determine the molecular mechanism by which metabolism of the halogenated inhalation anesthetic halothane causes toxicity in patients and operating room personnel. We are studying three inter-related aspects of this problem: First, the metabolic pathway by which formation of free radical metabolities of halothane leads to peroxidation of cell membrane phospholipids and how some of these peroxidized phospholipids may be converted to leukotriene B-4, a potent mediator of inflammation and activator of macrophages; second, the role of free radical metabolites or phospholipid oxidation products (such as leukotrienes) in activating hepatic lysosomes or in activating external macrophages to attack the cell membrane of the hepatocyte; third, one of the earliest observable signs of anesthetic toxicity in a liver cell, the loss of bound ribosomes from the rough endoplasmic reticulum.

Methodology

In our previous studies we have demonstrated that human metabolism of halothane is different from that in rats and monkeys. Therefore, we have developed the methodology to purify human cytochromes P-450 along with human cytochrome P-450 NADPH-reductase into phospholipid vesicle system that is capable of carrying out halothane metabolism like that in liver cells. Because of the well defined nature of this system, we are able to study the effect of each specific step of the metabolism of halothane on the surrounding membrane components. We have carried out the experiments involving metabolism of halothane, the formation of phospholipid hydroperoxides and the production of leukotrienes, in this reconstituted human cytochrome P-450 system. The metabolic products in these studies are isolated by high pressure liquid chromatography and analyzed by mass spectrometry.

We have used rat liver cells grown in monolayer cell culture for our studies of intracellular and extracellular damage that result from free radical metabolities of halothane and the resulting lipid peroxidation. It is well known that hypoxia induced in the experimental animal exacerbates liver damage due to halothane exposure. We have exposed hepatocytes in cell culture to hypoxia during the metabolism of halothane as well as during injury produced by addition of calcium ionophores and hydroperoxides. We have isolated the resident macrophage in the liver, Kupffer cells, and have added them to the hepatocyte monolayers in order to establish if they are activated and subsequently will lyse hepatocytes that are producing the chemoattractant anesthetic metabolites during halothane exposure. We have monitored the changes in intracellular structure of the hepatocytes by transmission electron microscopy, the attack of macrophages by scanning electron microscopy, and metabolite toxicity to hepatocytes by measuring their loss in ability to exclude the vital dye Trypan Blue as well as by increased leakage of cytosolic enzymes.

The studies of the detachment of ribosomes from the rough endoplasmic reticulum will be carried out by isolating microsomes from human liver cells; subjecting the microsomes to exposure to metabolites of halothane as well as to alterations in the oxidation reduction potential of the surrounding buffer as measured by a platinum electrode. Following treatment, the microsome suspension will be placed on a linear density gradient and centrifuged to separate the free ribosomes from those remaining on the human liver microsomes.

Progress & Accomplishments

The studies with the reconstituted human cytochrome P-450 system have provided the first definitive structure of the free radical metabolites of halothane and carbon tetrachloride, we have shown how these radicals bind to phospholipids, and we have demonstrated that they are a direct cause of phospholipid peroxidation. We have shown that leukotriene B-4 is produced in low yield when peroxidized arachidonic acid is allowed to react with human cytochrome P-450 in the absence of oxygen. Our studies with rat liver hepatocytes in cell culture have shown that hypoxia greatly increases the sensitivity of liver cells to the toxicity of calcium ionophores and hydroperoxides.

Significance

Our results demonstrate the importance of lipid peroxidation and hypoxia in the development of acute hepatic damage following exposure to halothane. Our studies with leukotrienes and Kupffer cells are giving an indication of how the immune system may be involved in liver damage. Our experiments are designed to examine the toxicity of halothane to patients and operating room personnel. However, halothane (2-bromo-2-chloro-1, 1, 1-trifluroethane) contains all three common halogens; the molecular mechanisms by which it causes liver toxicity may be common to many of the halogenated hydrocarbons that are found in the workplace and the environment.

Publications

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BREATH SAMPLING FOR INDUSTRIAL SOLVENT EXPOSURE

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Other Occupational Concerns
5 R01 OH01490-02
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$28,791 ($28,791 Cum)

Objectives

The overall goal of this project is to develop and demonstrate a method for sampling and analysis of trace compounds in exhaled air, that will be convenient for field surveys of large numbers of workers. Breath analysis has theoretical advantages over other methods of monitoring worker exposure to industrial solvents, but its utilization has been hindered by unreliable or impractical sampling technology. The proposed method uses an air-purifying respirator, with a special sampling cartridge added to the exhaled air port. A worker under examination would inhale contaminant-free air as usual, but his exhaled air would pass through the sampling cartridge which removes organic compounds and water vapor for subsequent analysis. The project consists of two stages: determination of cartridge sampling efficiency using controlled atmospheres simulating the properties of exhaled breath, and demonstration of the system in human volunteers exposed to controlled low levels of a commonly used industrial solvent.

Methodology

The sampling cartridge contains four layers of activated charcoal cloth placed upstream of sixty grams of molecular sieve granules. Organic compounds in exhaled air are adsorbed on the charcoal, which is later removed and analyzed by extraction and subsequent gas chromatography. Moisture in the exhaled air is collected on the molecular sieve and quantified gravimetrically; this provides an estimate of the volume of breath sampled. The sampling efficiency of these components was determined separately and then in combination. Controlled atmospheres containing toluene at concentrations between 1 and 10 ppm in air at 95% relative humidity were passed through the charcoal cloth at steady flow rates approximating human resting ventilatory rates. Sampling periods of up to one hour were employed. The cloth was then eluted in 10 ml of carbon disulfide for one hour and an aliquot of the resulting solution was analyzed following NIOSH recommended analytical methods.

The performance of the molecular sieve was evaluated by placing the sampling respirator on human volunteers whose minute volumes were determined simultaneously by an independent method. The volunteers performed exercise at selected levels to produce a wide range of exhaled volumes. The weight gain of the molecular sieve was compared with the measured minute volume over sampling periods of up to thirty minutes.

The combined sampling cartridge was then demonstrated in human volunteers under conditions meant to simulate realistic workplace conditions. Ten subjects were first exposed to toluene in filtered air at 40 ppm for four hours. (The current PEL is 200 ppm TWA; NIOSH recommends 100 ppm TWA). Between eighteen and twenty hours later their exhaled breath was sampled for thirty minutes using the modified respirator. This timing corresponds to taking a sample at the beginning of the next shift following exposure. After analysis as described above the resultant concentrations of toluene in the breath were compared with published data from similar clinical exposures where breath sampling techniques not appropriate for field use were employed.

Progress & Accomplishments

Recovery of toluene from the charcoal was consistently between 70% and 75% of the mass delivered to the cartridge at relative humidities above 90%. These represent very severe sampling conditions for activated charcoal, under which granular forms have shown very poor and inconsistent recoveries. The cloth form appears to give much better results in this application. At this level of performance, with modest gas chromatographic sensitivity, nanogram quantities of toluene can be quantitated in breath. Further, an excellent correlation was found between volume of breath exhaled by the subjects and weight gain of the molecular sieve, despite wide variation in ventilatory rate and breathing pattern. This method provides an accurate means of determining the sample volume, so that exhaled concentration may be calculated from the cartridge results.

Following experimental exposure to toluene vapor in air, the respirator sampler gave the following results: mean exhaled concentration 18 to 20 hours later was 0.1 ppm by volume, with a standard deviation of 0.025 ppm. These results are in good agreement with the only known published report of clinical exposure to similar toluene concentrations, indicating that the new sampling method gives results for toluene that are close to those from laboratory-based techniques.

Significance

If the performance for sampling other organic solvents is similar to that for toluene, a sample taken for thirty minutes or less at the start of a work shift will detect solvents present at concentrations in the range of 1 to 10 ppb. This level of quantitation would permit monitoring of exposures at 1/10th of the PEL for many important organic compounds.

The sampling respirator developed in the project resembles closely standard half face air-purifying respirators. The resistance to breathing is only slightly greater on exhalation and the added weight from the sampling cartridge in negligible. The cartridge design is simple and can be easily mass-produced. A sample can be taken in thirty minutes or less, and could be collected while on the job, if desired. The new method has considerable promise for field biological monitoring of volatile organic compounds on a large scale.

Publications - None to date

LEAD AND CADMIUM TOXICITY RELATED TO INDUSTRIAL EXPOSURE

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Other Occupational Concerns
5 R01 OH01522-02
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$80,999 ($148,757 Cum)

Objectives

Industrial exposure to cadmium and lead is a widespread and serious occupational problem. This proposal is directed toward evaluating the effects of these two metals, in single and combined exposure, in the human subject, and in an animal model, and with in vitro techniques. It is proposed that those workers at risk because of occupational contact with these two heavy metals be examined to determine if there are abnormalities of renal function that will serve as hallmarks of early toxic effects, prior to the development of established (and potentially irreversible) disease.

Methodology

The classical clearance technique has been utilized in both the human subject and the experimental animal. The evaluation of tubular transport function has also been assessed by employing the brush border membrane vesicle (BBMV) technique utilizing rat kidney tissue.

Progress & Accomplishments

A group of workers from a chemical plant located in Cleveland, Ohio were studied. In addition to the determination of the usual parameters of renal function, the subjects were investigated utilizing two new specialized techniques. The body burden of lead was evaluated with a standard three-day test in which lead excretion in the urine is promoted by infusion of the chelating agent, EDTA. These observations were compared to data obtained, in collaboration with investigators at the Brookhaven National Laboratory, utilizing an x-ray fluorescence technique to estimate tibial lead content. In addition, lead and cadmium content were determined employing a neutron activation technique. These findings were compared to blood and urine cadium levels. The results are as follows: 1) There is a direct linear correlation between tibial lead content and lead body burden as measured by the determination of urinary lead excretion following EDTA chelation. 2) There were few indications of overall renal functional abnormalities in this group of workers.

In animals given 2% lead in their drinking water, the clearance technique and BBMV transport were utilized to evaluate the effects of the led ingestion. While no animals have shown evidence of a phosphaturia or of alterations in glomerular filtration rate, BBMV studies did indicate a defect in transport at the proximal tubular level.

Progress & Accomplishments

With regard to the human experiments, these studies have verified that a new non-invasive technique can supplant the older methodology, involving the intravenous infusion of a potentially toxic chemical, in the assessment of whole body lead burden.

The animal studies point up the fact that early transport abnormalities due to lead may occur, which do not necessarily become evident as disturbances of glomerular filtration rate or abnormalities of overall excretion rate. However, carefully performed clearance studies, based upon information gleaned from the rat studies, could be designed, which would pick up these early defects in kidney function due to lead toxicity.

Significance

1) A new non-invasive test for the assessment of lead body burden has been developed which can replace older, invasive, cumbersome and potentially hazardous methodology. 2) Animal studies have pointed the way to the use of carefully designed clearance studies to pick up early toxic effects of lead, prior to the time when overall kidney function has been damaged.

Publications - None to date

EFFECT OF UNUSUAL WORKSHIFT ON DICHLOROMETHANE TOXICITY

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Other Occupational Concerns
5 R01 OH01531-02
12/01/83 - 11/30/85
$57,753 ($113,287 Cum)

Objectives

The major objective of this proposal is to examine the possibility that workers exposed to chemicals during an unusual workshift, 12 hours per day instead of the normal 8 hours per day, may be at greater risk than those on the usual workshift. Two major aims are being investigated. One is the development of a rodent model to determine if such a study can be done in experimental animals to mimic man. The second, related to the first, is whether dichloromethane demonstrates a greater toxicity when administered by inhalation during the unusual workshift using carboxyhemoglobin measurements as the endpoint for toxicity. Additional aims added during the course of the investigation are the use of aniline as a test compound with methemoglobinemia as an endpoint and the use of trichloroethylene with indicators of hepatotoxicity being serum enzyme measurements and microsomal mixed function oxidases.

Methodology

Animals, mice and rats, were exposed for either 8 hours/day or 12 hours/day to dichloromethane by inhalation. Carboxyhemoglobin measurements were made after single and repeated exposures. In some cases, the inhibitor pyrazole was used to prolong the half-life of dichloromethane by inhibiting its metabolism and in other cases, the animals were subjected to forced exercise. In the studies on aniline, similar methodology was used. In this case rats were exposed to varying concentrations of aniline and methemogolobin levels measured at appropriate time points. Aniline in fat and blood samples was analyzed by gas chromatography after repeated exposure. When trichloroethylene was the test compound, serum sorbitol dehydrogenase was determined as well as microsomal ethylmorphine demethylation and glucose-6-phosphatase activity. Chamber concentrations were monitored continuously using an infrared gas analyzer.

Progress & Accomplishments

Experiments were conducted to compare the effects of a 12-hour exposure schedule to those of an 8-hour schedule on the carboxyhemoglobin (COHb) formation resulting from dichloromethane (DCM) inhalation. Rats and mice were exposed to 200, 500, or 1000 ppm DCM for 8 hr/day for 5 days or 12 hr/day for 4 days. The effect of the unusual exposure schedule of COHb levels was not significant. The metabolic pathway for the formation of COHb appeared to be saturated even at the lowest concentration DCM. To examine the possible increase in the retention of inhaled DCM in the longer exposure schedule, single exposures for 8 and 12 hr were compared. The peak blood DCM level was dependent upon the DCM exposure concentration, but the half-life was independent of the duration of exposure and the concentration of DCM. The half-life of COHb in blood was prolonged by increasing the DCM concentration, but was not affected by the exposure period. Pyrazole treatment decreased the COHb level and increased the blood DCM level in rats exposed to DCM.

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When rats were placed in a cage which rotated at 7 rpm during the last 15 minutes of every hour of exposure, there was no difference immediately following exposure to 500 ppm dichloromethane or at one or two hours post-exposure between the carboxyhemoglobin levels or blood levels of DCM in these animals and those similarly exposed but without exercise. When the exposure level was increased to 1000 ppm and the rotation rate to 11 rpm, there was a slight decrease in the carboxyhemoglobin and DCM levels in the exercising animals immediately after exposure and at one hour post-exposure.

Experiments were conducted to examine the effects of altering the exposure schedule of rats from 8 hr/day for 5 days to 12 hr/day for 4 days on the toxicity resulting from the prior to and following the exposure, increased with the days of exposure at 50 ppm aniline. At 150 ppm aniline, the pre-exposure MetHb level increased with the days of exposure whereas the post-exposure MetHb levels appeared to plateau after the second exposure. The pre-exposure MetHb levels were significantly different between the two groups at 50 or 150 ppm aniline whereas the post-exposure MetHb levels were not. A residual MetHb level higher than the non-exposed control was observed in the 12 hr/day exposure group after a 3-day recovery period. There was an aniline concentration-dependent decrease in the hematocrit level when determined one week after the exposure started. Following a single exposure for 8 or 12 hr to 100 ppm aniline there was no difference in the maximum MetHb level, the peak aniline level in blood and fat, or the rate of aniline elimination from fat and blood. Repeated exposures to 100 ppm for 3 days did not produce a significant difference in aniline level in blood or fat between the 8 and 12 hr/day exposure groups immediately following the last exposure. However, the aniline level in blood was significantly higher in the 12 hr/day exposure group when determined on the next morning.

When groups of rats were exposed to 500 ppm trichloroethylene by inhalation, ethylmorphine demethylation was increased in the group exposed for 12 hours for one day but not for 8 hours/day. After one week of exposure hepatotoxicity was demonstrated to a greater extent in the 12 hour/day group than in the 8 hour/day group as determined by measurements of microsomal glucose-6-phosphatase activity.

Significance

The studies demonstrate that for chemicals with short half-lives, such as DCM, workers would not be expected to be at greater risk to their toxicity if encountered for 12 hours per day rather than the usual 8 hours per day. However, for compounds with long half-lives, such as aniline and trichloroethylene, alterations in permissible exposure limits may need to be considered.

Publications - Abstract(s) available

BENZENE TOXICITY IN BONE MARROW PRECURSOR CELLS

Daniel Wierda, Ph.D.	Other Occupational Concerns
Department of Pharmacology and Toxicology	5 R01 OH01542-03
West Virginia University Medical Center	09/30/83 - 08/31/86
Morgantown, West Virginia 26506	$71,480 ($208,089 Cum)

Objectives

The goal of this project is to examine the relationship between in vivo and in vitro toxic effects of benzene and its metabolites on bone marrow, hemopoietic cell proliferation and differentiation. Specifically, experiments are directed toward studying the interaction of the hemopoietic microenvironment with developing precursor B-lymphocytes and precursor granulocytes/monocytes after acute exposure to benzene or its metabolites.

Methodology

A coculture system that consists of adherent, bone marrow stromal cells and an agar overlayer of bone marrow precursor cells has been established to study how benzene or its metabolites alter stromal cell supported growth of precursor granulocytes/monocytes. A second culture system which involves the tandem use of liquid culture followed by agar culture was used to investigate how benzene or its metabolites affect maturation of bone marrow, precursor B-lymphocytes. Marrow cell suspensions were first depleted of mature surface IgM positive B cells by using antibody-coated Petri dishes. These cells were incubated in liquid culture for 48 hours after which time the number of precursor B-cells that have matured into surface IgM positive cells was assessed phenotypically with fluorescent antibody and functionally via an agar colony forming assay.

Progress & Accomplishments

Initially dose-response studies were undertaken to characterize the in vitro effect of benzene metabolites on adherent stromal cell colony formation. It was established that the TD_{50} (dose that reduced colony number by 50%) was 0.018 mM for benzoquinone, .0025 mM for hydroquinone 0.060 mM for benzenetriol, 0.125 mM for catechol and 0.190 mM for phenol. In addition, both hydroquinone and benzoquinone were shown to impair the ability of adherent stromal cells to support the hemopoiesis of granulocyte/monocyte colonies in agar. Further studies were done to determine if in vivo exposure of mice to benzene or its metabolites would result in similar damage to the stromal cell microenvironment. Both B6C3F1 and DBA male mice were given benzene (100 mg/kg) or phenol (100 mg/kg) intraperitoneally, twice a day 4 consecutive days. On day 5 bone marrow from the femurs and tibias was removed, plated in culture and assayed for the ability of stromal cells to proliferate and to support granulocyte/monocyte colony formation (hemopoiesis) in agar. Although stromal cell growth and proliferation were unaffected by the treatments, the ability of these stromal cells to support hemopoiesis was reduced to a greater degree among B6C3F1 mice than among DBA mice which paradoxially showed an increase in the ability to support hemopoiesis but no preferential effect among strains was evident. These results suggested that benzene, but not phenol, is metabolized differently between the two mouse strains and that bone marrow stromal cells of the hemopoietic microenvironment are sensitive targets for benzene or its oxidative metabolites.

To investigate the mechanism involved in stromal cell responses to *in vivo* benzene exposure, we have begun to test the hypothesis that benzene metabolites are affecting stromal cell secretion of regulatory factors such as colony stimulating factor or prostaglandin E_2 (PGE). Male B6C3F1 mice were given benzene (100 mg/kg), indomethacin (2 mg/kg) or a combination of benzene plus indomethacin was given one hour prior to benzene. On day 5 marrow cell suspensions were prepared from the mice. Benzene had no effect on bone marrow cell number, granulocyte/monocyte precursors or stromal cell colony number, but the ability of stromal cells to support hemopoiesis in agar was significantly decreased. In contrast, benzene had no effect on stromal cells taken from mice pretreated with indomethacin. This observation was subsequently corroborated by in *in vitro* experiments which demonstrated that indomethacin, which decreases PGE levels in stromal cell cultures, can ameliorate hydroquine induced toxicity.

We also have begun to address whether short-term exposure to benzene metabolites alters the ability of bone marrow precursor B-lymphocytes to mature into functional B-lymphocytes. Marrow cells from B6C3F1 mice were first depleted of mature surface positive IgM (B-cells) lymphocytes, exposed to 0.0001 mM hydroquinone for one hour and then plated in liquid culture. Pre-B (sIgM negative) cells were then allowed to mature in culture for 48 hours. Phenotypic and functional analysis of the cells detected a 36% decrease in the frequency of newly generated B-cells. These results demonstrated that although hydroquinone reduced the absolute numbers of B-cells formed, it did not inhibit the ability of the remaining precursors to mature into functional B lymphocytes. Whether this result is due to a selective effect on Pre-B cells or upon accessory stromal cells which support B-cell development is currently being investigated.

Significance

In summary, our research has demonstrated that bone marrow stromal cells, components of the hemopoietic microenvironment, are more sensitive targets of benzene or its metabolites than are hemopoietic precursors. This toxicity to stromal cells results in altered hemopoiesis or blood cell formation. In addition, our results point to a mechanism of toxicity which involves an imbalance in regulatory factor secretion (such as colony stimulating factor or PGE) by stromal cells following benzene exposure and that this toxic effect can be ameliorated by pretreatment with PGE synthesis inhibitors. Thus benzene may be capable of inducing blood dyscrasias or leukemic neoplasia via oxidative metabolites that interfere with the normal regulatory feedback network of the hemopoietic microenvironment.

Publications - Abstract(s) available

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Gaido K, Wierda D: In vitro effects of benzene metabolites on mouse bone marrow stromal cells. Toxicol Appl Pharmacol 76:45-55, 1984.

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CHROMIUM DISTRIBUTION AND TOXICITY IN MAMMALIAN CELLS

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Other Occupational Concerns
5 R01 OH01630-03
09/30/83 - 08/31/87
$84,690 ($245,094 Cum)

Objectives

To study the mechanisms of chromium toxicity and the intracellular distribution of chromium in vertebrate cells.

Tissue culture techniques can be used to advantage in the study of chromium genotoxicity, especially in regard to chromosomal aberrations and cell proliferation. Previously, we (Uyeki and Nishio, 1983) noted that in cultured CHO (Chinese Hamster Ovary) cells, the SCE (sister chromatid exchange) effects of Cr(VI) were observed at 0.01 - 0.03 uM, whereas the antiproliferative effects were observed at 1.0 - 10.0 uM. Thus, 30- to 100-fold differences in effective concentrations were observed between the two assays. Based on these concentration differences, we suggested a working hypothesis to probe further into the cell biology of chromium effects: the genotoxic effects are casually related to the antiproliferative effects in tissue culture systems.

Methodology

Several techniques will be utilized: tissue culture of cells, either from passaged cell lines (for example, CHO cells) or from primary embryonic tissue (for example, the chick limb bud cells), the HPLC of nucleotides and nucleic acids and digital image processing (DIP) of cytological specimens.

Progress & Accomplishments

Many DNA damaging agents such as genotoxicants, anticancer agents, mutagents or carcinogens increase SCE (sister chromatid exchange) frequencies in vertebrate cells. Chromium is one of these agents and we have shown that enhanced SCE frequencies occur at 10-30 nanomolar Cr(VI) in cultured CHO cells. Moreover, because other chromium-sensitive biological events such as the inhibition of cell proliferation occur at one micromolar, an effect that occurs at 30-100 fold higher concentrations, we suggested that the SCE effects are casually related to the antiproliferative effects. In order to probe the heuristic value of this notion, we selected the differentation of chondrocytes from chick limb bud mesenchymal cells as a suitable test system. We earlier showed that when stage 22-25 chick limb bud cells are cultured for 96 hr, they undergo the following sequence: activation of ADP-ribose transferase, DNA synthesis, cell proliferation, nodule formation, and proteoglycan synthesis (detected by Alcian blue staining and radioactive sulfur incorporation).

Our results on chromium (VI) genotoxicity to limb bud cultures are generally in accord with the notions of Cleaver and Morgan who recently suggested a hypothesis for the role of poly(APD-ribose) synthesis in damaged cells: DNA strand breaks set in motion a cellular response which results, among other events, in the activation of nucleases. Poly (ADP-ribosyl)ation is also a cellular response to DNA strand breaks by which several chromatin proteins become covalently modified for a brief period. The turnover of

ADP-ribosyl groups occurs in a matter of minutes. In those instances where enzyme activity has been directly measured, ADP-ribosylation inhibits enzyme activity. Poly (ADP-ribosyl)ation inhibits enzymes (such as the nucleases) that are activated promptly by alkylating agents and other genotoxicants. Thus, it appears that as there are cellular mechanisms to expand the initial scission of DNA strand breaks, there are some coincident cellular mechanisms to limit the degree of chromosomal damage. For instance, whereas DNA strand breaks set in motion a chain of nucleolytic events, some of which are the activation of nucleases, other events, such as poly (ADP-ribosyl)ation, by covalently attaching to these chromatin proteins, exert a damage-limiting influence (viz., inhibit these nucleases). In the continued presence of 1.0 uM or higher concentrations of Cr(VI), DNA strand continue to break; these breaks favor the balance of events toward nucleolytic action rather than repair of damage.

Significance

A principal concern about genotoxicity is its close correlation with carcinogenicity. There is an implied notion of a reciprocal relationship between differentiation and carcinogenicity; on the one hand, cells differentiate and, as a result, stop proliferating. On the other (carcinogenicity), cells begin to proliferate and, as a result, are no longer differentiated. Hence, to experimentally assess the effects of chromium in differentiating tissues may give us insight into the cellular mechanisms of chromium carcinogenicity. The micromass culture technique of Solursh, using dispersed cells obtained from chick limb buds, has been used as a model to study cell differentiation; mesenchymal cells from stage 22-24 chick limb buds differentiate into chondrocytes. We used this assay system to determine whether chromium (VI) can effect mesenchymal cells to differentiate into chondrocytes. A criterion for the sensitivity of such a system is the effective molar concentration of chromium (VI), relative to its effects on SCE and on cell proliferation. Our present studies indicate the following level of sensitivity to chromium (VI) effects:

1. Enhanced SCE effects	0.01 uM Cr(VI),
2. Inhibition of chondrocytic differentiation	0.3 uM Cr(VI),
3. Inhibition of cell proliferation	1.0 uM Cr(VI),

Thus, the sensitivity of cell differentiation to chromium effects, is intermediate between enhanced SCE effects and the inhibition of cell proliferation.

Publications

Nisho A, Uyeki EM: Inhibition of DNA synthesis by chromium compounds. J Tox Environ Health 15:237-244, 1985.

Uyeki EM, Nishio A: Antiproliferative and genotoxic effects of chromium on cultured mammalian cells. J Tox Environ Health 11:227-235, 1983.

Nakanishi S, Uyeki EM: Benzamide on chondrocytic differentiation on chick limb bud cell culture. J Embryol Exp Morph 85:163-175, 1985.

EVALUATION OF OCCUPATION LISTED ON ROUTINE DATA SOURCES

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Other Occupational Concerns
1 R01 OH01957-01
07/01/84 - 01/31/86
$36,833 ($36,833 Cum)

Objectives

The aims of this project are:

1. to estimate the comparability of useful occupation and industry calculated from a complete occupational history with the subject's own designation of usual occupation,
2. to compare each of these entries (calculated usual occupation and industry and self-designated occupation) with occupational entries of the death certificate and cancer registry abstract.

Methodology

The proposed research is intended to compare, for a sample of incident New Jersey cancer cases and controls, occupational data which have already been collected from three independent sources: personal interview, cancer registry abstracts, and death certificates. The information recorded on the death certificate and cancer registry abstract will be compared with the more accurate information collected from a lifetime occupational history obtained during personal interview.

Progress & Accomplishments

All data have been coded and formatted for comparability. Data analyses are nearing completion.

Significance

The long-term objective of this research is to suggest to epidemiologists and other researchers the most effective uses of routinely collected occupational data by describing how accurately these data sources characterize the usual occupation and industry of individuals. If the death certificate or cancer registry abstract often describe last occupation/industry or current occupation/industry, then the value of these sources for occupational epidemiologic studies will need to be reassessed.

Publications - None to date

IMPROVED PERSONAL PROTECTION AT HAZARDOUS WASTE SITES

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Other Occupational Concerns
5 R01 OH02066-02
09/28/84 - 08/31/86
$120,938 ($238,559 Cum)

Objectives

Goal 1. Investigation of the utility of FTIR spectroscopy performed with an attenuated total reflectance (ATR) attachment for the acquisition of spectra of hazardous waste materials having a variety of physical and chemical properties.

Goal 2. Investigation of the applicability of a variety of forward and reverse search algorithms used in combination with spectral libraries to the identification of components of the hazardous waste mixtures, and the sensitivity of these searches to the presence of minor components of mixtures.

Goal 3. The development of a compatibility testing scheme that utilizes FTIR analysis data, including guidelines on the use of such data to define personal protection strategies.

Methodology

All experiments were performed using a Nicolet 20 SX FTIR equipped with a 1280 computer, a 160 megabyte storage module, and a Harrick PLC S1M ATR system. Software development was based on adaptations to the programs PAIRS and LSFMIX using Fortran 77 and Macro language programming on the Nicolet 1280 computer.

All samples were prepared from pure Aldrich (Gold Label) solvents, or, in the case of actual hazardous waste samples, obtained from the U.S. EPA NEIC (Denver).

Progress & Accomplishments

The first step in the achievement of this objective was to define what is meant by "hazardous waste materials":

Matrices:

A. Mixture of hydrocarbons from n-decane through n-eicosane
B. # 2 fuel oil
C. Authentic waste oil from remedial action site
D. Mixture of acrylic enamel paint solvents
E. Synthetic acrylic enamel paint sludge
F. Authentic paint sludge from remedial action site

Compound classes (of target analytes):

1. Chlorinated, cyclic
2. Polychlorinated biphenyl
3. Chlorinated, aliphatic
4. Non-chlorinated, aromatic
5. Phenolic
6. Isocyanate
7. Organophosphate
8. Nitrile

Subsequent investigation found these definitions to be inadequate. Therefore, an investigation into the frequency of occurrence at hazardous waste sites of compounds of significance to health was initiated.

In addition, several hazardous waste samples were procured from the U.S. EPA and a variety of other sources. Some of these samples have been subjected to extensive analysis by other methods.

Goals 1-3

A Nicolet 20 SX - 1280 FTIR was acquired, installed in our laboratory, and all personnel trained in its use. Software was installed in the instrument, including forward search routines, the Aldrich medium resolution spectral library, the Aldrich high resolution spectral library (which is not commercially available), the Boolean logic program GRAB, and the artificial intelligence program PAIRS (which is not available for use on Nicolet 1280 computers except in our laboratory).

Goal 1

Progress into the investigation of the applicability of ATR for spectral acquisition is complete and is being addressed in a manuscript which will be submitted to Environ. Sci. Technol. during January, 1986. Questions of ATR versus transmission cells center on the questions of shifts of absorbance peak maxima and absorbance ratios. This project is central to our present effort.

Goal 2

This is the area of greatest accomplishment as of this time. Investigation of forward search routines was completed and reported in the AIHA Journal. Investigation of the program PAIRS for the identification of functional groups of principal constituents of simulated hazardous waste mixtures was completed. Application of PAIRS to the identification of the principal components of simulated hazardous waste mixtures was completed and reported in a publication accepted by Analytical Chemistry for publication in June 1986. Development of the program PAWMI is completed and has been submitted to Analytical Chemistry. The programs LSF and LSFMIX, as well as a variation on these programs written in our laboratory, were investigated for spectral curve fitting and quantitation. This effort is not yet ready for reporting.

Goal 3

The goal of incorporating these findings into a hazardous waste site personal protection strategy has not been addressed. However, since the grant has only been active for less than 14 months, it is not surprising that this last goal has not yet been addressed.

Significance

The above progress represents a very substantial portion of the work that can be accomplished in the laboratory towards investigating the utility of FTIR for the improvement of personal protection strategies at hazardous waste sites. The advances have been accomplished in analytical chemistry that will allow the industrial hygiene advances, but not without actual field testing. Proposals have been submitted to perform field testing of the method, and application of the findings to industrial hygiene during that field trial.

Publications

FTIR spectroscopy applied to hazardous weste: Preliminary test of material analysis for improvement of personal protection strategies. Am Ind Hyg Assoc J 46:181, 1985.

ANEMOMETRY RELATED TO WORKPLACE CONTAMINANT DISTRIBUTION

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Other Occupational Concerns
5 R03 OH01825-02
05/01/84 - 04/30/86
$11,802 ($30,621 Cum)

Objectives

This research examines the relationship between air circulation in a test room and the spatial and temporal distributions of a contaminant gas continuously released from a fixed point source. The aim is to develop empirical measures of ventilation, based on tracer gas techniques and anemometry, that can be used to estimate contaminant distributions and contaminant removal in ventilated spaces.

Methodology

Tracer gases have been widely used in indoor ventilation studies to observe flow patterns and the dispersal of contaminants. A residence time model is particularly appropriate for relating tracer gas measurements to local contaminant distributions and ventilation rates. The central feature of this approach is to characterize flows by the age or residence time of fluid elements, permitting an elegant and powerful analysis based on the statistical age distribution of the air at different points in the room. This technique provides results that are independent of the specific mixing mechanisms, and simultaneously measures the air distribution patterns and contaminant removal ability of the room ventilation system.

Local contaminant levels and age distributions are directly related to diffusive and convective transport by turbulent air movements. Measurements of the air velocity vector and its oscillations can provide point estimates of both transport mechanisms, so that anemometry may give rapid, useful predictions of parameters for a residence time model.

Contaminant distributions will be studied in a 5400 ft^3 test chamber under controlled ventilation conditions. A contaminant release will be simulated by injecting a passive tracer gas at a point in the chamber. The age distribution of the air will be obtained by recording the change in tracer concentrations with time with a computer controlled multi-port air sampler that collects air concentration data at several points (presently 16) in the room. Local contaminant distributions and age distributions are then calculated from these data by numerical integration. Air movement will be measured with a anemometer that can resolve the 3-dimensional wind vector in a typical room, and logged in the computer. The velocity data will be used to estimate the eddy diffusivity, air velocity vector, and to predict parameters of the local age distribution. The values predicted from the anemometry will then be compared to those measured with the tracer gas. Predicted and observed results will be compared for several ventilation conditions and source generation rates.

Progress & Accomplishments

We have completed the necessary modifications of the test chamber and air sampling system to prepare it for tracer studies of air mixing. In addition, a theoretical framework has been developed for interpreting the air velocity data and relating these measurements to parameters of the residence model. Some testing of the computer controlled tracer injector and sampler system has been done, indicating that the residence time model provides useful data on room ventilation and local contaminant levels. The local mean age seems to be a temporally stable temperature conditions, and room air flow rate.

Significance

The ability to estimate ventilation rates and predict contaminant levels at various room locations has obvious applications for evaluating the effectiveness of room ventilation systems, and provides and measuring index (i.e. air concentration) which is of primary interest to health scientists. The significance of this work arises from its longer-term potential to lead to a field deployable instrument and associated set of procedures for measuring ventilation in rooms, and determining how most efficiently to utilize or alter ventilation and source characteristics in a workplace.

Publications - None to date

ACUTE BIOCHEMICAL TOXICOLOGY OF ALIPHATIC NITRILES

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Other Occupational Concerns
1 R03 OH01897-01
07/01/83 - 12/30/84
$10,395 ($10,395 Cum)

Objectives

Aliphatic nitriles are commercially important, but toxic, organic chemicals. Each day, thousands of American workers are potentially at risk from nitrile poisoning. The acute toxicity of these nitriles has been attributed to the slow metabolic release of cyanide. In the case of succinonitrile, the reaction appears to be mediated via a two-step pathway firstly through hepatic oxidases and then by alcohol dehydrogenase. I propose to test other nitriles by: Specific Aim 1; establishing the effectiveness of alcohol dehydrogenase inhibitors to antagonize the acute toxicity of nitriles in mice, Specific Aim 2; testing multiple doses of alcohol dehydrogenase inhibitors with or without coadministration of thiosulfate as antidotal regimens in mice, Specific Aim 3; determining the ability of oxidized nitriles to serve as substrates for alcohol dehydrogenase in vitro, and Specific Aim 4; testing the ability of unusual microsomal enzyme inducers to potentiate the acute toxicity of nitriles in mice. My objective is to develop a better understanding of nitrile bioactivation and detoxification, and to suggest safer and effective antidotes for the management of human intoxication.

Methodology

Male CD-1 mice (Charles River Laboratories) weighing 25-30 gm were housed in groups of 3 to 6 in cages with pine shaving bedding. All solutions were prepared on the day of use and were usually injected in vehicle volumes of 1 ml/100 gm body weight. In all experiments involving attempts to modify nitrile toxicity the compounds were given to groups of 10 mice each that had received various pretreatments. In some cases treatments continued many hours after the nitrile. The mice were observed for 7 days and signs of toxicity, approximate time of death and final mortality were recorded. The data were analyzed statistically by chi square. A modified yeast alcohol dehydrogenase-nicotine adenine dinucleotide assay for ethanol (Sigma) was used to determine the initial rates of reaction of several selected hydroxy-substituted nitriles at various concentrations. These experiments were repeated using a preparation (Sigma) of horse liver alcohol dehydrogenase. Equilibrium constants were obtained by graphical analysis of the initial rates using doubly reciprocal plots. The amounts of cyanide released in each reaction mixture were determined by a modification of the Bruce method.

Progress & Accomplishments

I have used primarily n-butyronitrile, propionitrile and their respective alpha-carbon hydroxylated homologs, propionaldehyde cyanohydrin and lactonitrile, to examine the question of aliphatic nitrile bioactivation and toxicity in mice. Pretreatment of mice with the hepatic microsomal enzyme inducers pregnenolone-16a-carbonitrile, troleandomycin and isosafrole, or with the cytochrome P450-depleting agent cobaltous chloride, did not influence the mortality of mice given single doses of nitriles. Repeated injections of aspirin and indomethacin protected mice against death by nitriles, but the vehicle, dimethyl-sulfoxide, was equally effective. Aspirin or sodium salicylate in water had no effect. Repeated injections of 4-methylpyrazole or disulfiram also protected mice against death after nitriles. Most of the treatment regimens successful against the nitriles also protected against death due to the cyanohydrins. The cyanohydrins were more acutely toxic than their parent nitriles, produced death much more rapidly and resulted in the same toxic signs, suggesting that they are intermediates in the bioactivation pathway leading to free cyanide. The cyanohydrins appeared to serve as weak substrates for yeast alcohol dehydrogenase, however incubation of them with either yeast or horse liver alcohol dehydrogenase did not increase the rate of cyanide release over that in incubations where the enzymes were absent. This slow rate of cyanide release due to spontaneous hydrolysis cannot account for the rapid demise and high toxicity of the cyanohydrins in vivo, or for the efficacy of the treatment regimens which protected against death. It appears unlikely that prostaglandin synthetase or alcohol dehydrogenase are importantly involved in nitrile bioactivation. The same active process, however, appears to be responsible both for a-carbon hydroxylation and for the subsequent degradation of the resulting cyanohydrins to release free cyanide. It is far more efficient in mediating the latter reaction than the former.

Significance

The versatile chemical reactivity of the cyano functional group allows nitriles to be useful in a variety of industrial applications including the manufacture of plastics, synthetic fibers, resins, dyestuffs, vitamins and pharmaceuticals. They are also used as plasticizers, solvents, elastomers, agricultural insecticides and high-pressure lubricants. Nitriles are also toxic and some are teratogenic. Acute poisoning and death have been reported following inhalation of nitrile vapors and aerosols, or by dermal exposure to nitriles in the workplace. Many are at risk; NIOSH has estimated that 26,000 workers in the U.S. are potentially exposed each day to selected nitriles. The results of my research offer additional information concerning the bioactivation and toxicity of aliphatic nitriles and suggest several effective and safe antidote treatments for nitrile poisoning.

Publications - Abstract(s) available

DEVELOPMENT OF A NEW SAMPLING/ANALYSIS METHOD FOR OZONE

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Other Occupational Concerns
1 R03 OH01993-01
08/01/84 - 07/31/85
$6,528 ($6,528 Cum)

Objectives

To investigate the extrapolation of several liquid-phase ozonolysis reaction to solid phase reaction media. Candidate reactants included: 1,2-di-(4-pyridyl) ethylene, 4,4'-dimethoxystilbene and t-stilbene each coated in different solid substrates. If a stable stoichiometry could be established, the reaction(s) would be validated using NIOSH guidelines for sampling and analytical methods. Such a method would allow easier and more accurate field sampling for ozone in personal and area exposure situations.

Methodology

Each starting olefin was dissolved in a suitable solvent and coated on the following sorbents by use of a rotating evaporator or evaporation in a flask: glass wool (untreated), glass wool (DCMS treated), Florisil, Chromosorb T and W, silica gel, and Baker-10 extraction columns (silica gel bonded with octadecyl compound). Each sorbent was then packed in 3-inch glass tubes (6 mm OD, 4 mm ID) and exposed to a known concentration of ozone from a generator at given flow rates and sampling times. Ozone concentrations were verified with a chemiluminescent analyzer. The aldehyde produced by the reaction of the olefin with ozone was analyzed by GC with an appropriate column. The acceptance criteria for a reaction was that the mole percent of aldehyde produced (moles of aldehyde recovered/moles of ozone sampled) had to be within +/- 10 percent for all samples collected over a given sampling time and constant concentration. Sensitivity for the method called for the detection of a 0.05 ppm concentration of ozone over a one-hour sampling period. Finally, the sampling media had to be able to trap 100 percent of a 0.2 ppm ozone stream over an 8-hour sampling period.

Progress & Accomplishments

This project had addressed the original aims and has been terminated. The results obtained for the testing of the olefins is summarized as follows:

1. All three reactions worked best on Florisil and resulted in the production of the expected aldehydes. However, none of the reactions were stable in the solid phase and the yields of the aldehydes were unpredictable even with replicate samples from the same batch of prepared sorbent.
2. Results obtained from liquid phase reactions of the olefins could not be extrapolated to the solid phase given the stability of the reactant and reaction products.
3. Sample flow rate, olefin loading on the substrate, sampling duration, ozone concentration, time of sample elution and storage, and elution solvent had an effect on the reproducibility of aldehyde yield. These results were not conclusive since the reactant stability became the overriding concern.
4. Spontaneous oxidation of the olefins coated on solid supports was observed in bulk sorbents over time. Apparently oxygen in air would react with the olefins to give the corresponding aldehyde, which was also the

reaction product with ozone. The yields of aldehyde from sampling ozone in air with corrections made for blank amounts were much too large to be accounted for by ozone alone. Therefore oxygen in the ozone sample was a major interferent.

5. Aldehydes formed on the sorbent following reaction with ozone were not stable in a flowing media. The aldehydes would volatilize due to a fluid flow rates of 100-200 ml/minute.

6. Attempts to trap the volatilized aldehydes with other solid traps were unsuccessful and pointless as the amount of aldehyde trapped would not have been indicative of the amount of ozone sampled.

The olefins used in this study were not stable nor did they form stable reaction products with ozone in laboratory trials. Oxygen proved to be a major interferent, and such variables as sample time, storage time and flow rate introduced such variability that consistent mole percent yields of replicate samples with any of the olefins was impossible.

Significance

Future research along these lines would be pointless unless a much more stable olefin could be identified which would result in slower reaction times and lower product yields and a better bond between the substrate and the olefin. It is possible that having the olefins in a quasi-solid form would facilitate these outcomes. Near the end of this project, a study reported the binding of an olefin in a silicone-polycarbonate copolymer membrane and incorporated in a passive sampling device. Excellent precision (COV of 9%) and less than 1 percent interference from sulfur dioxide and nitrogen oxide was reported. A second possible approach would be through the use of a liquid-bound olefin. A recent development of a special porous plastic material which can retain 80 percent of its weight as a liquid bound inside the plastic may offer a possible solution. The porous plastic allows free passage or air into and out of the liquid phase while retaining the convenient properties of a solid. Although there might be problems with ozone reactivity and the type of plastic used, it might be possible to bind the olefin in a liquid phases within such a plastic and collect ozone as it diffuses into the membrane. The feasibility of this material for use in a passive monitor has been demonstrated.

Publications - None to date

SIZE DISTRIBUTION OF AIRBORNE LEAD AEROSOL IN INDUSTRY

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Other Occupational Concerns
5 R03 OH02044-02
11/01/84 - 10/31/86
$19,175 ($39,825 Cum)

Objectives

The overall goal of the proposed research is to assess whether a lead PEL based on size-differentiated sampling represents a more meaningful estimate of exposure. This would be accomplished by studying the impact of a variety of size distributions of lead aerosols on the predicted distribution of blood lead levels in workers exposed to lead.

Methodology

1. The lead aerosol size distribution in the workers' exposure is determined in selected operations in a battery manufacturing plant and in a brass foundry, using the Marple Series 290s personal cascade impactor.
2. The actual daily lead intake of a worker in a selected operation will be estimated with the aerosol size information obtained and with ICRP lung deposition model.
3. The Bernard model, as adapted by OSHA, for lead metabolism in the human body will be used to describe the accumulation of lead in the body and to estimate the blood lead level of a worker after a certain period of occupational exposure.
4. The probability distribution of blood lead levels of a given worker population will be estimated and compared with that predicted by OSHA.

Progress & Accomplishments

Three studies of this project were conducted. They are described as follows:

1. Field Evaluation of Particle Bounce.

This study helps determine the proper collection surface in the personal cascade impactor to be used in sampling in this project. The aim is to select a collection surface that minimizes the effect of particle bounce in the cascade impactor. Five types of collection substrates were evaluated for particle bounce in the laboratory with lead dioxide dust, and in the field with brass pouring fume and brass grinding dust. It is found that oil-saturated Teflon membranes have similar collection characteristics as the oil-saturated sintered metal, a surface for which the collection efficiency is presumed to be independent of particle loading. For subsequent sampling, we select oil-saturated Teflon membrane as the collection surface in the cascade impactor.

2. Predicted Effect of Aerosol Size Distribution on Blood Lead Distribution.

The size distribution measurements of lead aerosol determined from a brass foundry and primary lead smelter were used to simulate blood lead distributions applying a pharmacokinetic model developed by Bernard and adapted by OSHA. The predicted distribution of blood lead levels were determined using the actual size distribution of aerosol as compared to the blood lead levels predicted by OSHA.

In the furnace area of smelter and the pouring area of the foundry the predicted mean blood lead levels are higher than that predicted by the standard whereas in the smelters sintering and mixing operation the blood lead levels are less than that suggested by the standard. The data supports the conclusion that size selective sampling needs to be considered for incorporation into the OSHA lead standard.

3. Sampling in a Brass Foundry and A Battery Manufacturing Plant.

A minimum of 10 personal cascade impactor samples were collected in four operations each in two factories. The four operations in the brass foundry were cutting, grinding, pouring, and furnace. The four operations in the battery manufacturing plant were pasting, grid casting, cast-on-strap unloading and high-voltage welding.

Preliminary analysis shows that the size distribution and the total concentration of lead aerosol varies markedly according to the operation in the brass foundry whereas the differences are minimal in the battery plant. In the battery plant, the data show that lead aerosol in all four operations have MMDs greater than 10um and total lead concentrations much lower than observed in brass foundry operations.

Significance

In the second study described above, we have demonstrated that size selective sampling should be considered for incorporation into the OSHA lead standard. Since personal samples give more representative estimates of workers' exposure than the area sample does, a detailed analysis will be done on the personal samples collected to determine the magnitude of the difference in the predicted blood lead distributions based on a size selective sampling and on OSHA's assumptions. This research may enable the development of a lead exposure sampling scheme whose end result is better protection for lead exposure workers.

Publications

Hinds WC, Liu WV, Froines JR: Particle Bounce in a Personal Cascade Impactor: A Field Evaluation. Am Ind Hyg Assoc J 46(9):517-523, 1985.

FORMALDEHYDE MOLECULAR STATES IN THE GAS PHASE

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Other Occupational Concerns
5 R03 OH02045-02
09/28/84 - 08/31/86
$8,000 ($23,000 Cum)

Objectives

(1) Determination of the reaction efficiency for derivatization of polar organics by BSTFA in the gas phase. (2) Design and construction of a gas phase monomeric formaldehyde generator. (3) Analysis of reaction products of monomeric formaldehyde and water at a variety of temperatures for determination of the equilibrium constant for formation of methylene glycol.

Methodology

(1) BSTFA/DMF solution is reacted with vapors of polar organics and the trimethylsilylated derivatives are quantitated by capillary gas chromatography using flame ionization detection. (2) s-Trioxane undergoes acid catalyzed depolymerization at elevated temperatures in an anhydrous gas stream to produce monomeric formaldehyde. Gas stream is analyzed by chromotropic acid technique, pararosaniline technique and BSTFA derivatization for the determination of conversion efficiency of s-trioxane to monomeric formaldehyde. (3) Effluent of the monomeric formaldehyde generator will be reacted with water vapor in a gas stream to form methylene glycol in a dynamic dilution/reaction system. Downstream measurement of reactant and product concentrations will be utilized to determine the equilibrium constant for the reaction at several temperatures between 20 and 40 degrees centigrade.

Progress & Accomplishments

Objective 1 has been completed and a publication is presently being drafted. A series of alcohols, glycols and glycolic ethers gave essentially quantitative yields for gas phase reaction with BSTFA when compared with vapor pressure data available in the literature. Objective 2 is presently being pursued with completion expected in May 1986. Objective 3 will be completed subsequent to the completion of Objective 2.

Significance

(1) BSTFA has been shown to be a compound that quantitatively derivatizes reactive compounds in the gas phase. Trimethylsilylation is an established method for improving separation of polar organics in gas chromatography and with these results appears to be appropriate for analysis of gas phase polar organics. (2&3) Formaldehyde exists in a variety of combined forms with water and methanol. In order to understand the gas phase chemistry of this important chemical, gas phase monomeric formaldehyde must be generated so that it may be reacted with other compounds in order to determine the species that may be present in atmospheres where human exposures occur. Calculation of the equilibrium constant for the reaction between water and formaldehyde will allow approximation of compounds present in atmospheres (i) where human exposures occur; (ii) utilized for animal exposures in toxicological assessment; (iii) in the humid airways of respiratory systems where carcinogenic effects have been observed.

Publications - None to date

COMPLIANCE WITH OSHA HEALTH STANDARDS: METHODS AND CASES

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Other Occupational Concerns
1 R03 OH02135-01
09/28/84 - 09/30/85
$24,000 ($24,000 Cum)

Objectives

The major goal of the study was to address methodological issues in the cross-plant analysis of OSHA inspection data on ambient air levels of hazardous substances. The second goal was to analyze the OSHA inspection data for specific substances, to assess the validity of proposed strategies for analysis.

Methodology

We investigate the methodological issues posed by non-randomness of the industrial hygiene data in the first paper. We model three sources of non-randomness: decisions where to inspect; decisions where to take industrial hygiene samples; and decisions whether to report samples. Tests for the predicted non-reporting selection bias draw upon both quantitative and qualitative sources from fieldwork in 1984. We gathered data from the original files in two OSHA area offices in order to compare data in those files with that recorded in the MIS. We also interviewed industrial hygiene inspectors, supervisors, and area directors in the area offices as well as other regional and national officials to understand better industrial hygiene policies and practice.

In the second paper, we apply a variance components model to the asbestos data in the MIS to test whether the variance in sector effects is swamped by plant effects. This information is useful in determining the reasonableness of using the model for predicting average exposures in a sector.

Progress & Accomplishments

The distinctive aspect of the data collected for the study was that it allowed us to test the predictions about non-reporting selection biases. We found a high level on non-reporting (around 45% of all compliance samples taken for each area office were missing from the MIS.) Contrary to prediction, however, we found that the loss did not appear to substantially affect the distribution of exposures reported, conditional upon reporting occurring in a given establishment. The percentage of samples over the PEL which were not reported was smaller than the overall non-reporting rate. Because the measured compliance rate is so high, about 95% in both offices, the effect of this extreme tail phenomenon was very small. This selectively is not observed at the first, second, or third quartiles.

Further analysis of the predicted selection biases requires use of different data sets. To analyze selection bias in the inspection decisions, analysis of the full MIS is necessary. To analyze sampling bias, we plan in the future to compare the MIS data with independent sources of data about individual establishments (such as Health Hazard Evaluations).

However, with this method we cannot separate sampling and non-reporting bias. For this reason, our results in this study are critical to interpreting such future work.

Analysis of the variance components model gave varying estimates of the relative size of plant and sector variances across different sectors. Manufacturing sectors generally appeared to have larger sector variances than plant variances.

Both papers are currently in working paper form.

Significance

This project is an important first step in analyzing the usefulness of the MIS for analyzing workplace exposures. The MIS data set is important because it uniquely provides data on worker exposures across a wide range of industrial sectors. We are aware of no other source of data that spans such a wide range of plants. Additional empirical work needs to be done testing the predictions regarding selection bias, particularly in the sampling decision.

Publications - None to date

SAFETY AND SENSITIVITY: ETHICAL ISSUES IN IDENTIFYING AND PROTECTING HIGH-RISK PERSONS IN THE WORKPLACE

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and Geography
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Other Occupational Concerns
RII 8217297
08/01/84 - 10/31/85
$40,000 ($40,000 Cum)

Objectives

The central question addressed by this project was: How should society respond to the differential sensitivity of workers to technological hazards in the workplace? The following objectives were formulated to answer this question: 1. a review of scientific progress in conceptualizing and identifying differential sensitivity, considering the implications of these developments for policies and practices in the workplace; 2. an inventory and analysis of ethical issues posed by differential susceptibitiy to hazards on the job; 3. a detailed analysis of the alternative responses made in three cases--lower back injury, smoking linked with asbestos exposure, and lead exposure; 4. development of methodologies, based in theories of social justice, for assessing equity in occupational exposure to risk; 5. development of ethical guidelines and strategies that could incorporate project results in occupational health and safety policies and practices.

Methodology

The research tasks undertaken in pursuing the above objectives were: 1. a state-of-the-art review of recent developments bearing on the definition and identification of worker susceptibility to hazards, searching major data bases as well as scientific, medical, and trade journals; 2. an inventory of ethical issues, as identified in the literature, in practitioner experience, and in our case studies; 3. clarification of ethical issues by investigating relevant criteria (such as the source of sensitivity, who or what is protected. lift-stage at which identification occurs, nature of the indentification program, nature and predictability of the harm, nature of responses to differential sensitivity); 4. analysis of alternative responses using case studies--lower back injuries, smoking/asbestos, cotton dust exposure, alpha-1-antitrypsin deficiency, reproductive hazards--to explore the risk issues and the ethical problems involved in management responses; 5. the formulation of draft ethical guidelines for defining, identifying, and responding to differential susceptibility to occupational hazards.

Progress & Accomplishments

The objectives of this project have been largely realized. In some respects they have also become more ambitious than originally projected. The scientific and technological developments, and their implications, have been surveyed; a conceptual model of susceptibility has been developed and described; case studies, treating scientific, risk, and policy issues as well as alternative responses, have been completed for lower back pain, byssinosis and smoking, asbestos and smoking, reproductive hazards, and alpha-1-antitrypsin deficiency; ethical issues have been explored in four chapter drafts as well as a tentative set of ethical guidelines. Each case study identifies and evaluates the ethical problems associated with differential susceptibility but also attempts to reach "closure" by suggesting how management should proceed. A solid analytical base supports the general ethical guides which we have formulated. A final task, in process, is to develop a decision path, or flow chart, to direct the application of the guides.

These results have been presented at several scientific meetings and symposia, including a full double session on "Managing Differential Susceptibility in the Workplace" chaired by Roger Kasperson at the annual meeting of the Society for Risk Analysis in October 1985.

Significance

The primary usefulness of the results is two-fold: 1. to identify the types of ethical problems which arise and show how they may be analyzed, and 2. to provide guidelines for management which may be employed (or adapted) by those responsible for decisions. In addition, it alerts other interested parties, some directly affected (e.g., workers), of their rights and needed protection.

It is our hope that this will have clear significance as these problems emerge in occupational health settings.

The entire project will be brought together in a book, tentatively entitled Managing High-Risk Workers: Scientific, Ethical, and Policy Problems, with extensive and fully documented treatment of the issues.

Publications - None to Date

AWARD SUMMARY OF ALL ACTIVE GRANTS DURING FY85

	Competing Grants		Total Grants	
	No. of Awards	Amount of Awards	No. of Awards	Amount of Awards
Grants from FY85 Budget ($6.50M)				
Research Project Grants (R01)	15	$1,731,918	53	$5,646,152
Career Development Grants (K01)	1	$32,400	12	$367,325
Small Grants (R03)	9	$184,184	20	$319,595
Other Grants	1	$134,800	1	$134,800
Subtotal	26	$2,083,302	86	$6,467,872
Grants from FY84 Budget ($6.50M)				
Research Project Grants (R01)	4	$248,832	17	$1,578,560
Career Development Grants (K01)	1	$32,400	1	$32,400
Small Grants (R03)	5	$74,776	21	$405,263
Other Grants	4	$149,875	4	$149,875
Subtotal	14	$505,883	43	$2,166,098
Grants from FY83 Budget ($4.74M)				
Research Project Grants (R01)	0	$0	5	$279,543
Small Grants (R03)	6	$87,943	6	$87,943
Subtotal	6	$87,943	11	$367,486
Awards from all Years				
Research Project Grants	19	$1,980,750	75	$7,504,255
Career Development Grants	1	$32,400	12	$367,325
Small Grants	14	$258,960	41	$724,858
Other Grants	11	$372,618	11	$372,618
TOTAL	46	$2,677,128	140	$9,001,456

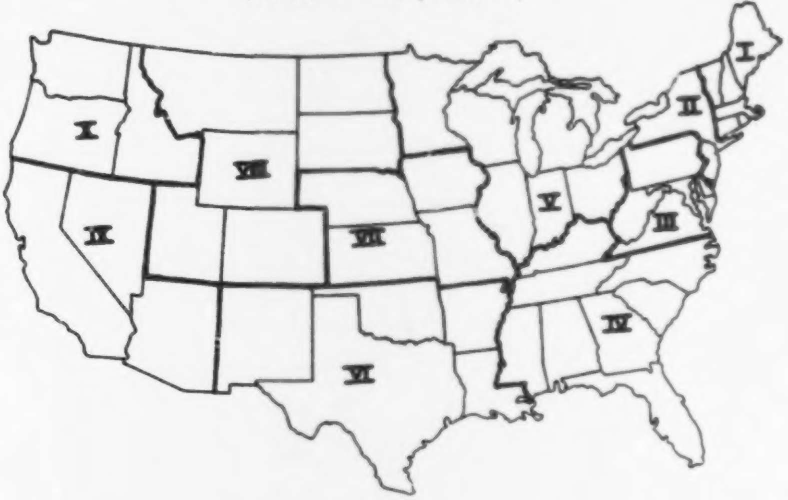
DISTRIBUTION OF GRANTS BY PROGRAM AREA

Program Area	Competing in FY85		Total Grants		
	No. of Awards	Amount of Awards	No. of Awards	Amount of Awards	Amt. Per.
Occupational Lung Diseases	3	$193,315	22	$1,260,873	14%
Musculoskeletal Injuries	1	$146,794	11	$708,464	8%
Occupational Cancers	2	$163,535	13	$1,001,217	11%
Traumatic Injuries	0	$0	2	$136,731	2%
Cardiovascular Diseases	1	$121,342	4	$161,071	2%
Reproductive Disorders	3	$281,948	14	$1,095,726	12%
Neurotoxic Disorders	6	$621,528	19	$1,301,700	14%
Noise-Induced Hearing Loss	1	$19,484	8	$480,451	5%
Dermatologic Disorders	3	$64,793	10	$350,753	4%
Psychologic Disorders	2	$202,159	3	$225,519	3%
Control Technology Research	3	$249,229	9	$592,286	7%
Respirator Research	0	$0	10	$873,296	10%
Other Occupational Concerns	1	$19,175	15	$813,369	9%
TOTAL	26	$2,083,302	140	* $9,001,456	100%

* Exceeds FY85 Budget of $6,501,000 because total includes all grants that were active at any time during FY85.

DISTRIBUTION OF GRANTS BY REGION AND STATE

140 GRANTS TOTALLING $9,001,456
(AMOUNTS IN $1000's)



	No.	Amt.	(Per.)
Region I	21	$1,426	(15.8%)
Connecticut	1	$58	(0.6%)
Massachusetts	14	$818	(9.1%)
New Hampshire	2	$119	(1.3%)
Rhode Island	1	$113	(1.3%)
Vermont	3	$317	(3.5%)
Region III	23	$1,117	(12.3%)
Maryland	10	$458	(5.0%)
Pennsylvania	9	$432	(4.7%)
Virginia	2	$128	(1.4%)
West Virginia	2	$99	(1.1%)
Region V	22	$1,511	(16.6%)
Indiana	1	$58	(0.6%)
Michigan	9	$705	(7.8%)
Minnesota	3	$228	(2.5%)
Ohio	8	$373	(4.1%)
Wisconsin	1	$147	(1.6%)
Region VII	9	$618	(6.8%)
Iowa	6	$406	(4.5%)
Kansas	2	$104	(1.1%)
Missouri	1	$108	(1.2%)
Region IX	16	$980	(10.9%)
Arizona	1	$112	(1.2%)
California	15	$868	(9.6%)

	No.	Amt.	(Per.)
Region II	18	$1,176	(13.1%)
New Jersey	1	$37	(0.4%)
New York	17	$1,140	(12.7%)
Region IV	12	$746	(8.3%)
Alabama	1	$65	(0.7%)
Florida	2	$85	(0.9%)
Georgia	3	$128	(1.4%)
North Carolina	5	$340	(3.8%)
Tennessee	1	$128	(1.4%)
Region VI	9	$809	(9.0%)
Arkansas	1	$115	(1.3%)
Louisiana	2	$140	(1.6%)
Texas	6	$554	(6.2%)
Region VIII	4	$146	(1.6%)
Colorado	4	$146	(1.6%)
Region X	5	$405	(4.5%)
Oregon	2	$71	(0.8%)
Washington	3	$333	(3.7%)

Foreign	1	$67	(0.7%)
Finland	1	$67	(0.7%)

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Research Project Grants (R01)

Grant Number	Principal Investigator	Current Funding	Year	Page
5 R01 OH 00535-09	Spencer, Peter S., Ph.D.	$111,742	84	133
5 R01 OH 00796-03	Ayer, Howard E.	$78,862	83	73
5 R01 OH 00823-06	Abou-Donia, Mohamed B., Ph.D.	$124,851	84	138
2 R01 OH 00835-07	Swartz, William J., Ph.D.	$59,864	85	108
5 R01 OH 00851-06	Spencer, Peter S., Ph.D.	$176,554	85	141
5 R01 OH 00923-05	Yu, C. P., Ph.D.	$58,351	85	10
2 R01 OH 00952-06	Scheving, Lawrence E., Ph.D.	$114,594	85	144
5 R01 OH 00978-06	Trudell, James R., Ph.D.	$211,985	85	254
3 R01 OH 00982-03S1	Bigos, Stanley J., M.D.	$190,230	84	52
5 R01 OH 01004-04	Middleman, Stanley, D.Eng.	$45,831	83	222
5 R01 OH 01035-03	Graham, William G., M.D.	$262,816	85	12
5 R01 OH 01122-05	Kauffman, Charles W., Ph.D.	$106,384	85	97
5 R01 OH 01151-02	Harlow, George E., Ph.D.	$64,355	83	14
5 R01 OH 01152-06	Henderson, Donald, Ph.D.	$123,163	85	177
5 R01 OH 01177-03	Baden, Jeffrey M., M.D.	$43,871	84	75
5 R01 OH 01245-03	Adams, Perrie M., Ph.D.	$62,548	84	110
5 R01 OH 01281-03	Juchau, Mont R., Ph.D.	$114,466	84	111
5 R01 OH 01301-03	Willeke, Klaus, Ph.D.	$71,790	84	235
5 R01 OH 01331-03	Bakale, George, Ph.D.	$109,050	84	77
5 R01 OH 01413-03	Peters, John M., M.D.	$189,478	84	113
5 R01 OH 01434-03	Ferm, Vergil H., M.D.	$108,702	84	115
5 R01 OH 01485-03	Liu, Benjamin, Ph.D.	$98,842	85	237
5 R01 OH 01490-02	Morgan, Michael S.	$28,791	83	256
5 R01 OH 01511-02	Matanoski, Genevieve, M.D.	$144,728	84	79
5 R01 OH 01520-03	Thysen, Benjamin, Ph.D.	$166,549	85	117
5 R01 OH 01522-02	Puschett, Jules B., M.D.	$80,999	85	258
5 R01 OH 01531-02	Carlson, Gary P., Ph.D.	$57,753	85	260
5 R01 OH 01533-02	Gierthy, John F., Ph.D.	$41,759	85	192
5 R01 OH 01542-03	Wierda, Daniel, Ph.D.	$71,480	85	262
5 R01 OH 01556-03	Menter, Julian, Ph.D.	$55,233	84	195
5 R01 OH 01557-04	Moss, Andrew P., Ph.D.	$72,582	85	80
5 R01 OH 01595-03	Hinds, William C., Sc.D	$62,011	85	239
5 R01 OH 01603-03	Nichols, Larry D., Ph.D.	$131,948	85	241
5 R01 OH 01605-03	Swift, David, Ph.D.	$69,061	85	17
5 R01 OH 01608-03	DuBois, Arthur B., M.D.	$57,754	85	243
5 R01 OH 01617-02	Raven, Peter B., Ph.D.	$94,583	85	245
5 R01 OH 01630-03	Uyeki, Edwin M., Ph.D.	$84,690	85	265
5 R01 OH 01644-02	Underhill, Dwight W., Sc.D.	$69,096	85	247
5 R01 OH 01646-03	Corn, Morton, Ph.D.	$119,866	85	249
5 R01 OH 01650-03	Merchant, James A., M.D.	$86,645	85	251
5 R01 OH 01910-02	Hemminki, Kari J., Ph.D.	$66,572	85	118
5 R01 OH 01928-02	Sulzer-Azaroff, Beth, Ph.D.	$30,347	85	99
5 R01 OH 01932-03	Perkins, Jimmy L., Ph.D.	$65,366	85	225
5 R01 OH 01955-02	Berman, Brian, M.D.	$33,039	84	198
1 R01 OH 01956-01	Mital, Anil, Ph.D.	$44,558	84	55
1 R01 OH 01957-01	Stemhagen, Annette, Dr.P.H.	$36,833	84	267

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Grant Number	Principal Investigator	Current Funding	Year	Page
5 R01 OH 01962-03	Schultz, Albert B., Ph.D.	$138,739	85	56
1 R01 OH 01968-01A1	Barnett, Rosalind C., Ph.D.	$169,759	85	216
5 R01 OH 01970-02	Hammad, Yehia Y., Sc.D.	$80,632	85	227
5 R01 OH 01972-02	De Caprio, Anthony P., Ph.D.	$43,581	85	147
1 R01 OH 02003-01A1	Abou-Donia, Mohamed B., Ph.D.	$175,874	85	149
5 R01 OH 02005-02	Harber, Philip I., M.D.	$80,761	85	252
5 R01 OH 02020-02	Sickles, Dale W., Ph.D.	$56,162	85	152
5 R01 OH 02021-02	Stedman, Donald, Ph.D.	$84,329	85	200
5 R01 OH 02024-02	Drury, Colin G., Ph.D.	$87,579	85	58
5 R01 OH 02027-02	Corbett, Michael, Ph.D.	$62,438	85	82
5 R01 OH 02029-02	Donham, Kelley J., D.V.M.	$208,958	85	19
1 R01 OH 02057-01	Kannel, William B., M.D.	$121,342	85	101
1 R01 OH 02065-01	Spencer, Peter S., Ph.D.	$131,882	85	154
5 R01 OH 02066-02	Levine, Steven P., Ph.D.	$120,938	85	268
5 R01 OH 02067-02	Swanson, G. Marie, Ph.D.	$238,477	85	84
1 R01 OH 02076-01	Carter, Dean E., Ph.D.	$112,239	85	21
1 R01 OH 02084-01	Walsh, Patrick R., M.D.	$146,794	85	60
1 R01 OH 02090-01	Settles, Gary S., Ph.D.	$39,649	84	229
1 R01 OH 02116-01	Johnson, Christopher M., Ph.D.	$60,105	85	23
1 R01 OH 02119-01	Draper, William M., Ph.D.	$69,031	85	86
1 R01 OH 02122-01	Fischbein, Alf, M.D.	$94,504	85	88
5 R01 OH 02128-02	Clark, William W., Ph.D.	$108,034	85	179
1 R01 OH 02132-01	Esmen, Nurtan A., Sc.D.	$96,059	85	230
1 R01 OH 02148-01	Cleary, Stephen F., Ph.D.	$108,768	85	120
1 R01 OH 02157-01	Letz, Richard E., Ph.D.	$157,787	85	156
1 R01 OH 02191-01	Boekelheide, Kim, M.D.	$113,316	85	121
7 R01 OH 02264-01	Kennedy, Thomas P., M.D.	$127,792	84	25
5 R01 OH 02317-02	Hamernik, Roger P., Ph.D.	$106,450	85	181
	Career Development Grants (K01)			
5 K01 OH 00002-02	Reiser, Karen M., M.D.	$32,400	85	27
5 K01 OH 00007-02	Shatos, Marie A., Ph.D.	$32,400	85	29
5 K01 OH 00011-02	Sokas, Rosemary K., M.D.	$19,440	85	103
5 K01 OH 00017-02	Guy, Richard H., Ph.D.	$30,605	85	201
5 K01 OH 00018-02	Kreiss, Kathleen, M.D.	$32,400	85	30
5 K01 OH 00019-02	Lantz, Robert C., Ph.D.	$27,801	85	31
5 K01 OH 00022-02	Cohen, Beverly S., Ph.D.	$32,400	85	33
5 K01 OH 00024-02	Caruso, Rita L., Ph.D.	$31,536	85	123
1 K01 OH 00028-01A1	White, Roberta F., Ph.D.	$32,400	85	218
1 K01 OH 00031-01	Obrams, Gunta I., M.D.	$32,400	84	90
5 K01 OH 00035-02	Prichard, Howard M., Ph.D.	$32,223	85	125
5 K01 OH 00042-02	Talbott, Evelyn O., Ph.D.	$31,320	85	183
5 K01 OH 00043-02	Letz, Richard E., Ph.D.	$32,400	85	157

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Grant Number	Principal Investigator	Current Funding	Year	Page
	Small Grants (R03)			
1 R03 OH 01677-01	Christiani, David C.	$14,071	83	35
5 R03 OH 01684-02	Kriebel, David, M.S.	$13,132	85	37
1 R03 OH 01704-01	Comstock, George W., M.D.	$19,736	83	158
5 R03 OH 01705-02	Cole, Dean A., M.S.	$18,644	84	127
5 R03 OH 01713-02	Snyder, Carroll A., Ph.D.	$17,980	84	129
5 R03 OH 01715-02	Ip, Michael Po Chee, Ph.D.	$24,750	85	39
5 R03 OH 01718-02	Gerhardt, Kenneth J., Ph.D.	$22,500	84	185
5 R03 OH 01737-02	Orchard, Susan M., M.D.	$21,405	84	203
5 R03 OH 01755-02	Marras, William S., Ph.D.	$22,630	84	62
5 R03 OH 01756-02	Tepper, Allison L., M.S.	$7,985	84	41
5 R03 OH 01758-02	Wilder, David G., M.S.	$21,988	84	64
5 R03 OH 01761-02	Spencer, Peter S., Ph.D.	$28,125	84	160
5 R03 OH 01763-02	Lapadula, Daniel, Ph.D.	$22,478	84	162
1 R03 OH 01794-01	Moreland, Rebecca R.	$21,468	83	66
5 R03 OH 01795-02	Wu, Joseph M., Ph.D.	$25,674	84	92
1 R03 OH 01799-01	Fidler, Anne T., M.S.	$19,125	84	165
5 R03 OH 01802-02	Freivalds, Andris, Ph.D.	$12,650	85	68
5 R03 OH 01825-02	Spear, Robert C., Ph.D.	$11,802	85	270
1 R03 OH 01826-01A1	Shalat, Stuart L., M.S.	$24,450	85	167
5 R03 OH 01827-02	Magee, Carol A., M.P.H.	$5,080	85	131
5 R03 OH 01829-02	Casali, John G., Ph.D.	$19,500	85	187
5 R03 OH 01830-02	Guy, Richard H., Ph.D.	$19,590	84	205
5 R03 OH 01833-02	Thompson, David C., M.S.	$19,600	84	94
1 R03 OH 01850-01	Reist, Parker C., Sc.D.	$6,445	83	43
5 R03 OH 01852-02	Armstrong, Thomas J., Ph.D.	$6,000	85	69
5 R03 OH 01856-02	Moseley, Pope L., M.D.	$20,700	84	45
5 R03 OH 01865-02	Kreiss, Kathleen, M.D.	$22,966	84	47
5 R03 OH 01867-02	Robins, James M., M.D.	$13,194	84	105
1 R03 OH 01893-01	Agnew, Jacqueline, M.P.H.	$18,028	84	168
1 R03 OH 01897-01	Kaplita, Paul V.	$10,395	83	272
5 R03 OH 01898-02	Seegal, Richard F., Ph.D.	$25,028	84	170
1 R03 OH 01904-01	Syme, Leonard S., Ph.D.	$7,095	84	107
5 R03 OH 01905-02	Nolan, Robert P., B.A.	$8,775	85	48
1 R03 OH 01906-01	Silverstein, Michael, M.D.	$15,828	83	71
1 R03 OH 01990-01A1	Kowal, Charles D., M.D.	$20,804	85	208
1 R03 OH 01993-01	Blehm, Kenneth D., Ph.D.	$6,528	84	274
5 R03 OH 02038-02	Sun, True-Jenn, M.S.	$2,362	85	173
1 R03 OH 02044-01	Froines, John R., Ph.D.	$19,175	85	276
5 R03 OH 02045-02	Utterback, David F., Ph.D.	$8,000	85	278
5 R03 OH 02071-02	Brix, Kelley A., M.D.	$23,360	85	220
1 R03 OH 02091-01	Rheins, Lawrence A., M.D.	$22,387	85	210
1 R03 OH 02095-01	Dusenbery, David B., Ph.D.	$16,941	85	175
1 R03 OH 02098-01	Guernsey, Judith R., Ph.D.	$20,971	85	50
1 R03 OH 02101-01	Flynn, Michael R., Sc.D.	$18,370	85	231
1 R03 OH 02135-01	Jones, Carol A., Ph.D.	$24,000	84	279
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1 R43 OH 02136-01	Hughes, Dominic W., Ph.D.	$50,000	84	190
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